FOOD ORAL PROCESSING AND NUTRITION THROUGH THE LIFESPAN

EDITED BY: Paula Midori Castelo, Elsa Lamy and Ana Carolina Mosca
PUBLISHED IN: Frontiers in Nutrition

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-264-9
DOI 10.3389/978-2-88971-264-9

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FOOD ORAL PROCESSING AND NUTRITION THROUGH THE LIFESPAN

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Citation: Castelo, P. M., Lamy, E., Mosca, A. C., eds. (2021). Food Oral Processing and Nutrition Through the Lifespan. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88971-264-9

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frontiers in Nutrition

EDITORIAL
published: 29 June 2021
doi: 10.3389/fnut.2021.702724

Editorial: Food Oral Processing and Nutrition Through the Lifespan

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Keywords: food oral processing, mastication, nutrition, eating behavior, saliva

Editorial on the Research Topic

Food Oral Processing and Nutrition Through the Lifespan

A balanced healthy diet is recognized as essential to prevent several non-communicable diseases, such as diabetes, hypertension, cardiovascular diseases or even some types of cancer. The need to promote shifts to healthier diets is even more relevant in the actual context, where obesity rates are increasing worldwide. Effective nutritional strategies, aimed at promoting healthier eating habits are warranted, from young to old ages. But for this purpose, a good comprehension of the factors involved in food preferences and choices is required.

Food oral processing comprises the sequence of transformations that food undergoes inside the mouth and will influence food sensory perception and food digestion. In oral processing, the food structure is first deformed and degraded by the forces applied by the teeth and soft tissues, including the tongue. Following, the fragments formed upon chewing are mixed with saliva, producing a bolus that can be safely swallowed. During this dynamic and synchronized process, the continuous interactions between oral structures, saliva and food produce the multiple sensations that are processed by humans into sensory perception. Moreover, the way the different food structures are broken and the food pieces are mixed with saliva affects (favoring or limiting) the biological availability and absorption of nutrients.

Masticatory performance influences the amount and type of food eaten, and, consequently, nutrition. An association between reduced chewing performance and obesity has been reported in the literature, with a higher consumption of soft high-fat and high-refined carbohydrates foods being associated to a reduced masticatory function. Masticatory function may vary according to several factors, among which oral health, age and sex. This can be particularly important in critical periods, such as the childhood, when food habits are defined, and at old age, where the compromised masticatory function will affect nutritional status. There are reports of better masticatory performance in normal-weight than in overweight children, with poor masticatory performance being also related with being underweight. For the elderly, masticatory difficulties resulting from teeth loss can lead to the risk of malnutrition. The design of new foods, able to overcome these problems, is a necessity to guarantee adequate nutrition.

Food oral processing has a major role in flavor perception and the participation of saliva in this process has been increasingly noticed in recent years. The role of this fluid in oral sensory perception started to be recognized in the case of astringency. Although the exact mechanism is still not completely known, this oral tactile sensation results from the interaction between salivary proteins with astringent molecules, disrupting the lubricating properties of saliva. Astringency usually results in food rejection. The way food is broken and mixed with saliva will affect the availability of astringent compounds to interact with salivary proteins and, consequently, affecting astringency intensity and food acceptance. Moreover, a relationship between salivary constituents

Edited and reviewed by:
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *29 April 2021*
Accepted: *03 June 2021*
Published: *29 June 2021*

Citation:
Lamy E, Mosca AC and Castelo PM (2021) Editorial: Food Oral Processing and Nutrition Through the Lifespan. Front. Nutr. 8:702724. doi: 10.3389/fnut.2021.702724

and basic tastes has been observed, as well as an influence of this fluid in the way the volatile molecules are released during food breaking by mastication. This suggests that the way food is mixed with saliva will influence food acceptance and the consequent development of dietary habits.

The present Research Topic provides a collection of high-quality manuscripts presenting different aspects of food oral processing and its interaction with sensory perception, eating behavior and nutrition at different ages. This issue is composed of 9 manuscripts, including 7 original research articles, one review and one perspective article from Asia, Europe, and South America.

The article from Marquezin et al. presents evidence about how morbid obesity is related with oral health and nutritional patterns, with individuals with higher body mass indexes presenting poorer dietary habits and behaviors. Possebon et al. investigated the effect of implant-retained mandibular overdenture in oral parameters and quality of life, concluding that the impact of oral rehabilitation on masticatory function should consider the facial morphology.

The relationship between saliva and food oral perception is also present in this Research Topic. Schwartz et al. review compiled the different works that describe how diet influences saliva antioxidant capacity and how the latter influence food perception. Okawa et al. presented data suggesting that chewing strokes and saliva flow rate are associated with the concentration of aroma released through the retronasal route.

The importance of texture for food acceptance and nutritional intake can be found in two original articles, from Tournier et al. and Schwartz et al. The first work assessed how the type of food presentation (puree, small pieces, etc.) modulates further texture acceptance in 4–36-month-old children, while the study of Schwartz et al. was designed to investigate the role of food presentation (texture) in satiation and subsequent intake in schoolchildren.

In line with the need of new food products that guarantee nutritional quality through lifespan, three different articles focus on the importance of food oral processing to achieve acceptance. Amoah et al. compared breads of increasing nutrient quality and Tejada et al. evaluated the effect of reducing the amount of salt in the acceptance of Iberian chorizo. Both studies showed the possibility of changing products to healthier options. De Lavergne et al. gave the perspective from industry point of view of providing benefits for various target populations. The authors highlight how scientific understanding about food oral processing is important for product development, even more when specific population groups are considered. Besides presenting the different methodologies that are generally used for assessing food oral processing, this article focuses on the current tendency of an increasing demand for plant-based products, what illustrates the relevance of deeper, multidisciplinary and integrated knowledge about the processes occurring in the mouth.

Overall, the studies presented in this Research Topic focus on different aspects of food oral processing, considering different age-group populations, and showing the relevance of food transformation in the mouth in the context of a healthier nutrition.

AUTHOR CONTRIBUTIONS

EL, AM, and PC proposed the scope and concept of the present Research Topic, invited researches, contributors, handled the submissions, and approved the final version. PC was the main corresponding Editor. EL prepared the main draft of this Editorial, which was reviewed by AM and PC. All authors contributed to the article and approved the submitted version.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 20 November 2020
doi: 10.3389/fnut.2020.589510

Oral Health and Nutritional Characteristics of Adults With Morbid Obesity: A Multivariate Analysis

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The relationship between oral health and nutritional aspects are complex, especially in individuals with chronic diseases and comorbidities, such as morbid obesity. Thus, the aim of the present study was to identify oral health and nutritional-related patterns in 113 individuals, aged 19–68 years (92 females), seeking treatment for morbid obesity. Sociodemographic variables and medical records were examined, in addition to the consumption of fruit, vegetables, candies, and processed foods. Measures of body mass index, neck, waist and hip, caries experience (DMFT index), Community Periodontal Index (CPI index), and salivary physicochemical aspects were gathered. Aspects of oral health-related quality of life and symptoms of dry mouth were evaluated by means of Oral Health Impact Profile (OHIP-14) and Xerostomia Inventory-XI questionnaires. K-means cluster analysis and, subsequently, comparisons between clusters (one-way ANOVA) were performed ($\alpha = 5\%$). Three clusters were generated: Cluster 1 (labeled "Young"; $n = 77$) was characterized by younger participants with higher BMI, who reported the use of distractors while eating, the smallest number of meals/day, and who consumed sweetened drinks and processed food the day before. Cluster 2 (labeled "Diabetic individuals"; $n = 12$) was characterized by older participants with the highest proportion of diabetic participants (100% were diabetic; 73% insulin users), lower BMI, higher DMFT index and OHIP-14 and xerostomia scores, and who reported having consumed fruit and vegetables the day before. Finally, Cluster 3 (labeled "Poor periodontal health"; $n = 24$) was characterized by participants with the worse periodontal condition (higher CPI), and lower salivary flow, pH, and buffer capacity. Cluster 1 and 2 were the groups that showed higher demand for nutritional and dietetic counseling, because of the poor eating behavior and higher serum glucose levels, respectively. On the other hand, Cluster 2 and 3 showed the higher demand for oral rehabilitation and dental treatment because of the loss of teeth and worse periodontal condition, respectively, besides the need for dietetic counseling. This sample of individuals with morbid obesity showed very unique oral-health and nutritional characteristics and special needs patterns that should

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Eating Behavior, a section of the journal Frontiers in Nutrition

Received: *30 July 2020*
Accepted: *09 October 2020*
Published: *20 November 2020*

Citation:
Marquezin MCS, Chaves-Júnior SC, Rasera I Jr, Pacheco ERP, Gavião MBD, Lamy E and Castelo PM (2020) Oral Health and Nutritional Characteristics of Adults With Morbid Obesity: A Multivariate Analysis. Front. Nutr. 7:589510. doi: 10.3389/fnut.2020.589510

be identified to adjust or change unhealthy habits, thus improving the assistance of this condition.

Keywords: morbid obesity, oral health, comorbidities, saliva, nutrition

INTRODUCTION

Obesity is considered a multifactorial and chronic condition that results in excess fat storage due to biological, behavioral, and environmental factors (1). According to the World Health Organization (2018) (2), 13% of adults have obesity (BMI of 30 to <40 Kg/m^2) in the world, which is associated with comorbidities such as type 2 diabetes mellitus, cardiovascular diseases, hyperlipidemia, sleep apnea syndrome, respiratory diseases, neoplasms, endocrine disorders, periodontal disease, psychosocial problems, among others (3). The prevalence of morbid obesity, characterized as BMI of 40 Kg/m^2 or higher, has also increased over the last 30 years (4). In the United States, an increase in the percentage of adults with morbid obesity was observed from 5.7% in 2007 to 7.7% in 2016 (5). In Brazil, an upward trend in morbid obesity prevalence was also observed, with women showing higher rates (1.3% in 2006 and 1.9% in 2017) than men (0.9% in 2006 and 1.4% in 2017) (6).

A worse quality of life is observed in people with excess of weight, relative to physical and mental health impairments, in addition to social problems (7). Obesity and oral health may share similar causal and behavioral mechanisms (8), mainly related to the diet. Previous studies reported an association between obesity and several oral diseases, such as dental caries (9), periodontal disease, tooth loss (7, 10, 11), and xerostomia (defined as the subjective perception of oral dryness) (9, 12).

In the previously mentioned studies, authors observed a higher number of tooth losses and higher frequency of periodontal disease in individuals with obesity (10, 13), pointing out their greater demand in health services and nutritional counseling. Considering the morbid obesity, there is a lack of such information and, thus, it is essential to deepen the knowledge about oral health characteristics of these individuals and their special needs, in order to ensure the better assistance, especially in individuals with morbid obesity.

The hypothesis of the study was that even among individuals with similar anthropometric status and seeking treatment for morbid obesity, many oral and nutritional health-related aspects may vary, given the multifactorial nature of these aspects; thus, when designing health care therapies, it is crucial to understand the specific needs of each group. In this sense, the aim was to perform an exploratory study using cluster analysis to identify oral health and nutritional-related patterns in adults with morbid obesity.

MATERIALS AND METHODS

Study Design

This is a cross-sectional study approved by the Ethical Research Committee of the Federal University of São Paulo (Protocol No. 1201/2017). All the individuals read and signed an informed consent form to take part in the study.

Sample

The study included a convenience sample of 113 adults, aged between 19 and 68 years with morbid obesity, who were evaluated just before starting a dietary program prior to gastroplasty surgery in the Bariatric Clinic of Piracicaba (SP, Brazil), between the years 2018 and 2019. Of them, 92 were females.

The inclusion criteria were individuals with morbid obesity (BMI of 40 Kg/m^2 or higher) of both sexes, with at least 20 natural teeth or who use dental prosthesis. The exclusion criteria were individuals presenting epilepsy, cancer, rheumatoid arthritis, bucco-dentofacial diseases or traumas, tobacco use, illicit drugs, Sjögren's syndrome, systemic lupus erythematosus, sarcoidosis, alcoholic beverage, and extensive tooth loss.

Anamnesis and Interview

Data collection was performed by means of a structured questionnaire applied during an interview by one of the authors (MCSM). The following data were investigated: date of birth, age, declared ethnic group (black/white/mixed), marital status, educational level, family income, weight, height, use of chronic medications, and any diseases or health conditions (hypertension, diabetes mellitus, dependent insulin), in addition to dental history. Measures of fasting glucose, uric acid, calcium, high density lipoprotein (HDL), low density lipoproteins (LDL), and total cholesterol laboratory analysis were gathered from medical records.

The qualitative assessment of food consumption habits was performed using a brief questionnaire proposed by the Brazilian Ministry of Health (14), which identifies the eating behavior and the consumption of healthy foods like fruit, vegetables, meat and beans, and unhealthy foods such as sausages, artificial juices, soft drinks, instant noodles, cookies, snacks, and sweets (processed and ultra-processed food). Participants answered questions regarding the consumptions of these items in the day before interview (yes/no). Questions also covered the habit of watching TV, using the computer and/or cell phone during the meal and the number of meals per day (day to day).

The Brazilian-validated version of the Oral Health Impact Profile (OHIP-14) was applied during the interview (15) and provided a comprehensive measure of the self-reported dysfunction, discomfort and disability attributed to the oral condition. This consisted of 14 items divided in 7 domains (functional limitation, pain, psychological discomfort, physical disability, psychological disability, social disability, and handicap). For each OHIP-14 item, participants were asked how frequently they had experienced the impact of that item. Responses were made on a 5-point Likert scale: 0 "never," 1 "hardly ever," 2 "occasionally," 3 "fairly often," 4 "very often."

OHIP-14 total scores, ranging from 0 to 56 points, were obtained by summing the responses on all 14 questions (items). Higher scores imply poorer oral health-related quality of life and thus, lower satisfaction.

Xerostomia was measured using the Xerostomia Inventory XI. Items were scored on a five-point unidirectional rating scale that rated the frequency of experiencing dry mouth symptoms from "Never" to "Very often." The scores range from 11 (no xerostomia) to a maximum of 55 (severe xerostomia) (16).

Clinical Examination

Physical examination was carried out at the clinic by a trained examiner (MCSM) and included measures of body mass index (BMI kg/m^2), neck, waist, and hip circumferences. Neck circumference was measured using a flexible ruler at the thyroid cartilage level (17). Waist circumference was measured to the nearest centimeter between the iliac crest and the lower rib (18) and hip circumference is measured at the height of the largest horizontal diameter. All measurements were performed twice and the mean was considered as the final value.

The oral examination was also performed at the clinic, in a private room, using a clinical mirror, probe and mouth retractors, according to the World Health Organization recommendations (19). Caries experience was evaluated by assessing the number of decayed, missing, and filled permanent teeth (DMFT). Periodontal status was assessed using the Community Periodontal Index (CPI), which has three indicators: gingival bleeding, calculus, and depth of the periodontal pockets; the possible scores are: 0- Healthy; 1- Bleeding after probing; 2- Calculus; 3- Pocket 4–5 mm; 4- Pocket 6 mm or more. The highest CPI score for each sextant was recorded from six teeth-indexes (16, 11, 26, 36, 31, and 46). Each tooth-index was carefully examined by a trained and calibrated examiner (SCCJ), using a mirror and WHO probe with a 0.5 mm ball tip (19) and applying a force of about 20 g (as recommended by the methodology during training).

Evaluation of Salivary Parameters

Stimulated saliva (SS) was collected from participants chewing on 0.3 g of an inert and tasteless material (Parafilm, Merifeld, USA) for 5 min into a cooled tube, in the morning, with all of them having refrained from eating, drinking or brushing their teeth for a minimum of 2 h before collection.

Salivary pH was determined immediately after collection, using a portable pH meter (Orion 3 Star Benchtop, Thermo Electron Corporation, USA). After calibration, the electrode was immersed in a Falcon tube containing saliva for 30 s for measurement. Further, buffer capacity was measured according to the methodology described previously (20, 21).

Taste Sensitivity

The evaluation of taste sensitivity was conducted using an adaptation of a validated methodology (22), called three-drop-method which uses four concentrations of each basic tastes (salty, sweet, sour, and bitter). In the present study, only the lowest concentration of each stimuli was used: salty–sodium chloride (0.016 g/mL), sweet–sucrose (0.05 g/mL), acid–citric acid (0.0125 g/mL), bitter–quinine hydrochloride (0.0001 g/mL), which were administered in a dropper (three drops) on the back of the tongue, with 1 drop of the tastant solution and 2 drops of distilled water, in order to verify the sensitivity to the lower concentrated tastants, which is near to the limit threshold.

The order of presentation of the tests was drawn for each individual. The participant should choose, for each of the tests, one of the four options: sweet, salty, bitter, or acid (sour), with no time limit for the test (forced choice). Between each test, individuals were instructed to rinse their mouths with mineral water to avoid residual taste that could confuse them. For each test correctly identified, the volunteer received 1 point, and the incorrect answers, either for not being able to identify the flavor or for having confused it with another flavor, do not add points (maximum of 4 points).

Statistical Analysis

Statistical analysis was performed using SPSS 26.0 software considering an alpha level of 5% by an Applied Statistics Spec (PMC). Descriptive analysis consisted of means, standard deviation, and percentages.

Firstly, hierarchical cluster analysis using farthest neighbor method for calculating distances between clusters was performed to obtain the dendrogram and analyze the range of clusters for further running K-means analysis, which was performed to identify groups of participants with similar oral health and nutritional-related variables. After *Z*-score transformation, the analysis included the following variables: age, sex, BMI, diagnostic of diabetes, insulin therapy, salivary parameters, taste sensitivity, oral health parameters, OHIP-14 and xerostomia scores, and food consumption behavior aspects. The final number of clusters was based on the interpretability and reliability of the cluster solution, and the differences between clusters were assessed by *F*-test for clustering validation (it is important to mention that F tests should be interpreted only for descriptive purposes, as the clusters were chosen to maximize the differences between each case and the other clusters).

Further, One-way ANOVA and Bonferroni's post-test were used to compare the clinical characteristics and aspects of oral health-related quality of life (OHIP-14 scores) and xerostomia scores between clusters.

RESULTS

The study included 113 adults between the ages of 19 and 68 years with morbid obesity. K-means cluster analysis generated three reliable and meaningful clusters of individuals, varying significantly according to the oral health-nutritional-related aspects of the participants. **Table 1** shows the final cluster centers (*Z*-scores cluster-variables means) which defined the three clusters gathered from multivariate analysis. According to the taxonomy description, Cluster 1 (labeled "Young"; $n = 77$) was characterized by younger participants with higher BMI, who reported to use distractors while eating, having the smallest

TABLE 1 | Final cluster centers (means of *Z*-scores) of the oral-nutritional-related variables (important differences which identify the clusters are colored).

	Cluster 1 Young	Cluster 2 Diabetic individuals	Cluster 3 Poor periodontal health	F-test
Number of cases	77	12	24	
Age	−0.3281	1.3656	0.3698	23.920
Sex	0.0435	−0.1642	−0.0576	0.271
Diabetes	−0.2785	1.9694	−0.0912	48.901
Insulin intake	−0.2761	2.5352	−0.2761	131.060
Body mass index	0.1083	−0.3607	−0.1626	1.559
Salivary flow	0.2487	−0.2736	−0.7696	10.555
Salivary pH	0.2091	−0.1282	−0.7303	8.024
Salivary buffer capacity	0.1406	0.1101	−0.5893	4.577
Taste sensitivity	0.0625	−0.0183	−0.1889	0.574
Periodontal condition	−0.2272	−0.1035	0.8366	11.633
DMFT index	−0.3029	1.0615	0.4669	15.342
OHIP-14	−0.1431	0.9911	0.0049	6.842
Xerostomia score	−0.0542	0.9314	−0.2530	6.180
Distractors use while eating	0.2824	−0.6426	−0.5889	10.878
Number of meals	−0.2965	0.6162	0.6861	12.946
Fruit consumption	−0.1827	0.6606	0.27588	4.908
Vegetable consumption	−0.0677	0.6615	−0.0584	2.470
Sweetened drink consumption	0.1577	−0.2222	−0.40425	3.324
Candy consumption	0.0704	−0.2964	−0.0901	0.768
Processed/ultra-processed food consumption	0.1488	−0.5036	−0.2574	3.126

DMFT, decayed, missing, and filled teeth; OHIP-14, Oral Health Impact Profile.

number of meals/day, and who consumed sweetened drinks and processed food the day before examination.

Cluster 2 (labeled "Diabetic individuals"; $n = 12$) was characterized by older participants with the highest proportion of diabetic subjects (100%; 73% insulin users), lower BMI, higher DMFT index, OHIP-14, and xerostomia scores, and who reported having consumed fruit and vegetables the day before. Finally, Cluster 3 (labeled "Poor periodontal health"; $n = 24$) was characterized by participants with the worse periodontal condition (higher CPI), and lower salivary flow, pH and buffer capacity. On the other hand, the sensitivity to the lower concentrations of sweet, salt, acid, and bitter tastes did not significantly contribute for clustering individuals.

Table 2 shows the characteristics of the clusters according to sociodemographic and clinical aspects. Sociodemographic aspects such as schooling (>8 years) and percentage of individuals who reported as ethnic group "white" were similar between groups. Among the measures of fasting serum glucose, uric acid, calcium, HDL, LDL, and total cholesterol, only fasting serum glucose level was different between clusters ($p < 0.001$; *eta* partial$^2 = 0.22$; power = 0.99), with a mean higher than 150 mg/dL in the cluster 2 labeled "Diabetic individuals." This cluster also showed the higher DMFT index; a closer look at the DMFT index revealed that the component "missing" contributed most to this difference (means: 1.9, 10.8, and 5.2 for Cluster 1, 2 and 3, respectively).

As mentioned above, Cluster 3 labeled "Poor periodontal health" also showed the most severe periodontal disease (on average, this cluster achieved a maximum grade of 2.7 ± 0.9), the higher mean number of decayed and filled teeth and the lowest means of salivary flow, pH, and buffer capacity (**Table 2**).

The impact of oral health on quality of life was evident in Cluster 2 ("Diabetic individuals") (**Table 3**), probably because of the large number of missing teeth as shown in **Table 2**. Besides, symptoms of xerostomia were also worse in individuals of Cluster 2; OHIP-14 and Xerostomia inventory XI total score of Cluster 2 were significantly higher than that found in the other clusters (mean 25.1 ± 9.5, $p = 0.002$, *eta* partial$^2 = 0.10$; mean 23.9 ± 5.9, $p = 0.003$, *eta* partial$^2 = 0.11$, power >80%, respectively) (**Figures 1**, **2**). The largest differences were perceived in the need to interrupt meals because of the oral conditions (OHIP-14, question 9) and difficulties when swallowing certain foods (Xerostomia inventory, question 7), emphasizing the perception of discomfort and difficulties that these individuals encounter during meals.

DISCUSSION

The relationship between oral health and nutritional status are complex and multifactorial, especially in individuals with chronic diseases and comorbidities, as morbid obesity. This relationship is bidirectional, as some systemic conditions may exacerbate oral symptoms of pain and discomfort, while oral

TABLE 2 | Description of the demographic and clinical characteristics of the clusters.

		Cluster 1 Young	Cluster 2 Diabetic individuals	Cluster 3 Poor periodontal health
	Number of cases	77	12	24
Age	Mean (±SD)	34.4 (8.8)	50.8 (8.2)	41.9 (8.7)
Sex (females)	%	83	75	79
Declared ethnic group (black/white/mixed)	%	17/52/31	18/55/27	13/58/29
Marital status (married or cohabiting couple)	%	51	58	74
Schooling (>8y)	%	97	100	96
Income (number of min wages)	Mean (±SD)	2.3 (1.7)	1.9 (1.1)	3.1 (2.6)
Body mass index (Kg/m^2)	Mean (±SD)	47.8 (9.1)	41.1 (2.1)	45.6 (8.0)
Neck circumference (cm)	Mean (±SD)	40.0 (4.0)	41.2 (4.3)	39.0 (3.9)
Waist-hip ratio (cm)	Mean (±SD)	0.6 (0.2)	0.6 (0.2)	0.6 (0.2)
Diabetics / Insulin users	%	9 / 0	100 / 73	17 / 0
Number of meals/day	Mean (±SD)	3.1^A (1.0)	4.0^B (1.2)	4.2^B (0.9)
Fasting glucose (mg/dL)	Mean (±SD)	101.8 ^{A} (19.4)	157.7^B (51.7)	128.2^A (58.5)
Uric acid (mg/dL)	Mean (±SD)	5.8 (1.1)	5.6 (1.4)	5.6 (1.8)
Calcium (mg/dL)	Mean (±SD)	9.4 (0.4)	9.5 (0.4)	9.4 (0.4)
High density lipoprotein (mg/dL)	Mean (±SD)	44.1 (11.2)	41.8 (3.5)	49.0 (11.2)
Low density lipoproteins (mg/dL)	Mean (±SD)	103.5 (30.8)	102.7 (38.8)	107.6 (37.7)
Total cholesterol (mg/dL)	Mean (±SD)	176.8 (33.3)	181.2 (42.0)	182.5 (41.5)
Stimulated salivary flow (mL/min)	Mean (±SD)	1.2^A (0.6)	0.9^{AB} (0.6)	0.7^B (0.4)
Stimulated salivary pH	Mean (±SD)	7.4^A (0.4)	7.2^{AB} (0.4)	7.0^B (0.6)
Buffer Capacity	Mean (±SD)	4.7^A (1.1)	4.6^{AB} (1.3)	3.9^B (0.6)
Community Periodontal Index	Mean (±SD)	2.1^A (0.6)	2.2^A (0.7)	2.7^B (0.9)
DMFT index	Mean (±SD)	8.3^A (6.1)	17.6^B (6.0)	12.3^B (4.7)
Component D (decayed)	Mean (±SD)	0.5 (0.8)	0.5 (0.7)	0.7 (0.9)
Component M (missed)	Mean (±SD)	1.9^A (2.7)	10.8^B (7.7)	5.2^C (6.8)
Component F (filled)	Mean (±SD)	5.8 (4.9)	6.1 (5.0)	7.5 (4.8)

A ≠ B in the same column ($p < 0.05$; One-way ANOVA; Bonferroni's post-test).
DMFT, decayed, missing, and filled teeth.

problems may interfere in eating and masticatory behavior (23). The multivariate analysis applied in the present study identified three clusters, named "Young," "Diabetic individuals," and "Poor periodontal health," with unique characteristics and special needs that should be recognized to better assist this condition. A higher number of females composed this convenience sample, probably because de frequency of obesity is higher in females and about 80% of patients who undergo gastroplasty surgery in Brazil are women (24).

Cluster 1 labeled "Young" included younger participants with higher BMI, who reported to use distractors while eating, have a lower number of meals per day and consumed sweetened drinks and processed food the day before examination. In the last years, the dietary patterns have been characterized by an increase in the consumption of high energy density foods, in which those rich in fibers have been replaced by products rich in fats and sugars, with a high level of processing and very low nutritional quality (25). These ultra-processed foods contain little or no whole food and are ready or almost ready for consumption and, therefore, easily accessible and convenient especially for the youth. They are combined with the use of additives, to make them durable and hyper-palatable, being consumed in a higher frequency in lower-middle- and upper-middle-income countries over the years (26). The consumption of this type of food has been shown to be a risk factor for obesity in adolescents and adults (27, 28) and the literature shows that individuals with obesity at a young age tend to remain with this condition throughout their lives (29).

The findings also corroborate a previous study which also noted that distractors, such as reading a printed text or using a smartphone, increase the total calories consumed compared to meals without distractors (30). The same is observed when watching TV (31, 32), and the reasons for this harmful effect seem to be related to distraction and satiety impairment (30, 33). Moreover, it was recently reported that children used to watch TV during meals have lower preferences for vegetables, comparatively to the ones that do not have this distractor during meals (34), what may be also a cause of higher energy intake. Palatable foods, rich in fats and sugars, usually have softer consistency and low content of fibers, preventing food to stay longer inside the mouth to be chewed and, again, impairing satiety (35). Cluster 1 showed the lower demand for oral rehabilitation, probably because it was composed by younger individuals; even so, dietary and nutritional counseling are useful for this groups to improve the quality of what is being ingested, thus preventing negative consequences for oral and general health in the future.

TABLE 3 | Oral health-related quality of life and xerostomia scores description according to clustering groups (mean and SD).

		Cluster 1 Young	Cluster 2 Diabetic individuals	Cluster 3 Poor periodontal health	*p*-value (power)
OHIP-14	Have you had trouble pronouncing any words because of problems with your teeth, mouth or dentures?	0.4 (0.9)	1.1 (0.9)	0.6 (1.2)	0.154 (0.40)
	Have you felt that your sense of taste has worsened because of problems with your teeth, mouth or dentures?	0.5^{A} (0.9)	1.4^{B} (1.5)	0.6^{AB} (1.1)	0.029 (0.66)
	Have you had painful aching in your mouth?	1.3 (1.1)	2.1 (0.8)	1.9 (1.1)	0.025 (0.69)
	Have you found it uncomfortable to eat any foods because of problems with your teeth, mouth or dentures?	1.3 (1.2)	2.2 (1.5)	1.3 (1.3)	0.088 (0.49)
	Have you been self-conscious because of your teeth, mouth or dentures?	1.7 (1.4)	2.7 (1.4)	1.9 (1.5)	0.106 (0.46)
	Have you felt tense because of problems with your teeth, mouth or dentures?	1.1^{A} (1.5)	2.3^{B} (1.6)	1.0^{AB} (1.2)	0.031 (0.65)
	Has your diet been unsatisfactory because of problems with your teeth, mouth or dentures?	0.7^{A} (1.2)	2.0^{B} (1.8)	1.1^{AB} (1.4)	0.011 (0.78)
	Have you had to interrupt meals because of problems with your teeth, mouth or dentures?	**0.5^{A} (0.9)**	**1.8^{B} (0.9)**	**0.5^{A} (0.8)**	**<0.001 (0.99)**
	Have you found it difficult to relax because of problems with your teeth, mouth or dentures?	0.8 (1.2)	1.7 (0.9)	0.8 (1.0)	0.058 (0.56)
	Have you been a bit embarrassed because of problems with your teeth, mouth or dentures?	1.1^{A} (1.5)	2.3^{B} (1.6)	1.4^{AB} (1.8)	0.049 (0.59)
	Have you been a bit irritable with other people because of problems with your teeth, mouth or dentures?	0.7 (1.3)	1.3 (1.0)	0.5 (1.1)	0.198 (0.34)
	Have you had difficulty doing your usual jobs because of problems with your teeth, mouth or dentures?	0.4^{A} (0.8)	1.5^{B} (1.1)	0.4^{A} (1.1)	0.003 (0.88)
	Have you felt that life in general was less satisfying because of problems with your teeth, mouth or dentures?	0.6^{A} (1.1)	2.1^{B} (1.5)	0.9^{A} (1.5)	0.001 (0.94)
	Have you been totally unable to function because of problems with your teeth, mouth or dentures?	0.3 (0.7)	0.7 (1.3)	0.4 (0.9)	0.142 (0.40)
	OHIP-14 total score	11.2^{A} (11.1)	25.1^{B} (9.5)	13.0^{A} (12.0)	0.002 (0.92)
Xerostomia Inventory-XI	I sip liquids to aid in swallowing food	2.1 (1.5)	1.6 (1.2)	1.9 (1.6)	0.624 (0.13)
	My mouth feels dry when eating a meal	1.1^{A} (1.3)	2.4^{B} (1.7)	1.0^{A} (1.4)	0.013 (0.76)
	I get up at night to drink	2.0 (1.5)	2.7 (1.8)	1.8 (1.4)	0.344 (0.24)
	My mouth feels dry	1.9 (1.4)	2.5 (1.4)	2.0 (1.4)	0.486 (0.17)
	I have difficulty in eating dry foods	1.0 (1.3)	2.0 (1.7)	1.2 (1.5)	0.099 (0.47)
	I suck sweets or cough lollies to relieve dry mouth	0.9^{AB} (1.3)	1.9^{A} (1.6)	0.6^{B} (1.1)	0.025 (0.68)
	I have difficulties swallowing certain foods	**0.6^{A} (0.9)**	**1.9^{B} (1.7)**	**0.3^{A} (0.7)**	**<0.001 (0.97)**
	The skin of my face feels dry	0.99^{A} (1.4)	2.3^{B} (1.9)	0.8^{A} (1.4)	0.011 (0.78)
	My eyes feel dry	0.8 (1.2)	1.4 (1.9)	0.9 (1.4)	0.376 (0.22)
	My lips feel dry	1.9 (1.5)	3.0 (1.2)	1.7 (1.6)	0.048 (0.59)
	The inside of my nose feels dry	1.6^{A} (1.5)	3.0^{B} (1.4)	1.7^{A} (1.3)	0.003 (0.88)
	Xerostomia Inventory-XI total score	15.0^{A} (8.9)	23.9^{B} (5.9)	13.3^{A} (9.6)	0.003 (0.88)

$A \neq B$ in the same column ($p < 0.05$; One-way ANOVA; Bonferroni's post-test); the parameters for which $p < 0.001$ were highlighted in bold.
BMI, body mass index; OHIP-14, Oral Health Impact Profile.

Cluster 2, which included diabetic individuals (whose 73% were insulin users), also showed a great need for nutrition counseling and oral health treatment because of the higher serum glucose levels, loss of teeth, and dry mouth symptoms. The literature shows some evidence on the relationship between diabetes and oral health status, such as the higher risk of severe periodontal insertion losses that may lead to tooth loss; also, diabetic individuals may also show hyposalivation, which can be associated with the xerostomia symptoms reported. It is interesting to note that no statistically significant decreases in the amount of saliva collected was observed; however, saliva collection occurred under stimulation and it is possible that at rest these individuals present lower salivary flow rates. In addition, changes in the oral microbiota, healing difficulties, abscesses and hyperplasia associated with the pathophysiology of diseases or their drug treatments were mentioned (36). Diabetes probably influences periodontal disease because of vascular abnormalities, neutrophils dysfunction, abnormalities in collagen synthesis and genetic factors predisposition (37) and the severe periodontal insertion losses increases the possibility of tooth loss. Previous findings showed that diabetic patients with mild to moderate periodontal disease suffer a more negative impact on quality of life than healthy ones or those with only gingivitis (38, 39). The present results corroborate a previous one (40) which concluded that tooth loss impacts on quality of life independently of the instrument used to measure quality of life

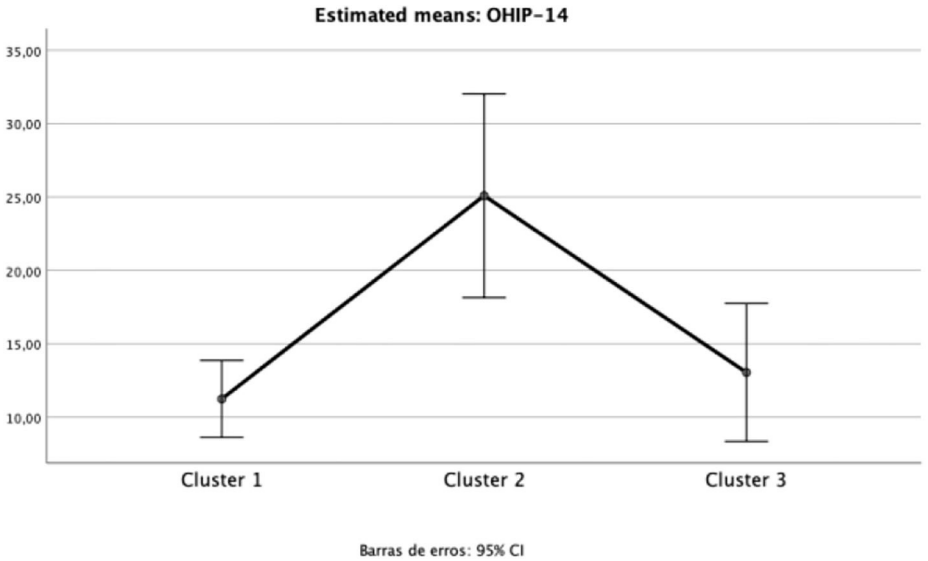

FIGURE 1 | Mean and 95%CI of Oral Health Impact Profile (OHIP-14) total scores for each cluster (one-way ANOVA $p < 0.05$; *eta* partial squared = 0.11; power = 92%).

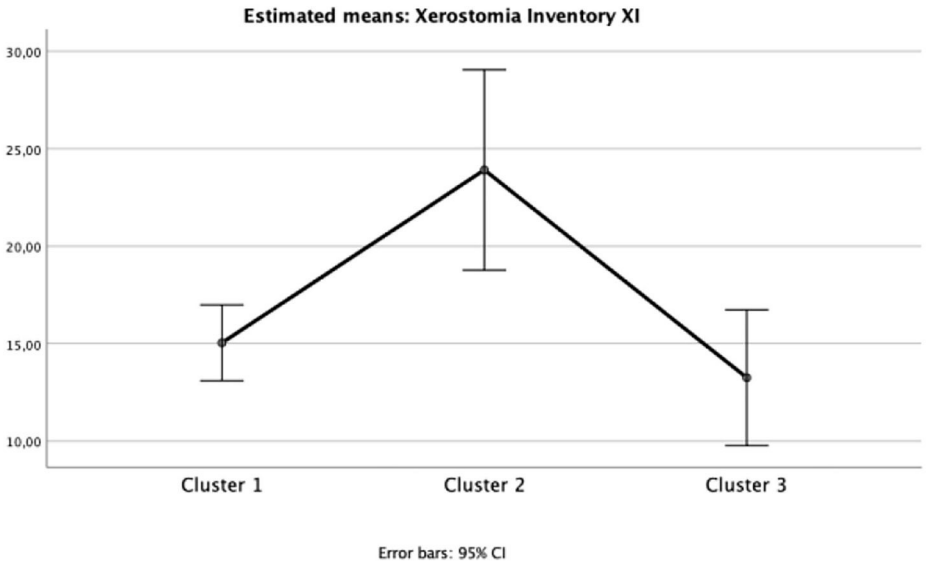

FIGURE 2 | Mean and 95%CI of Xerostomia Inventory XI total scores for each cluster (one-way ANOVA $p < 0.05$; *eta* partial squared = 0.10; power = 88%).

or the social context and, in general, studies have shown that the absolute number of teeth as well as their relative position in the mouth are associated with impairments on the oral health-related quality of life (40, 41). According to the results gathered by OHIP-14, it seems logical that the interruption of meals, as well as the exclusion of certain foods from the diet are related to the reduced number of teeth. In the social sphere, the fewer number of healthy teeth is associated with absenteeism at work and to the feeling of social disadvantage (36). The loss of natural teeth significantly reduces the masticatory performance and, thus, acts as a significant barrier in relation to the food choice, and edentulism or partial edentulism are important risk factors for malnutrition (23). The inability to chew or properly grind foods encourage the exclusion of high-fiber foods and favors the consumption of overcooked food (42) and foods with softer consistency, thus potentially affecting the glycemic control.

There are some possible causes for qualitative and quantitative changes in saliva secretion in diabetic patients, as well as the feeling of dry mouth and the reported difficulties in swallowing certain foods and taste changes. Glycosuria, caused by mild hyperglycemia, results in fluid loss and dehydration of the body

and can lead to decreased salivary secretion. Also, structural pathologies of complex etiologies can occur in the salivary glands, decreasing their saliva production (43–45). Combined, xerostomia complaints and tooth loss have a great impact on masticatory function and nutrition and, for this reason, it is of utmost important that these patients receive proper nutritional and dietary guidance and dental rehabilitation (46).

Finally, Cluster 3 ("Poor periodontal health") showed the poorer oral health status, with the most severe periodontal disease, higher number of decayed and filled teeth and lower means of salivary flow, pH, and buffer capacity, and which mostly needs advice on oral health and dental treatment. Periodontitis can negatively affect inflammatory pathways, also affecting the systemic health, which may increase the risk of other diseases such as cardio-metabolic disorders (47). Individuals with obesity appear to be at higher risk for the development of periodontitis as both share a low-grade inflammatory state, and a positive association between them was found in different populations (48). However, the literature has not confirmed a cause and effect relationship between these conditions until present (49).

It is possible that excess weight is a determinant of hyposalivation (50), which is defined as an objective measure of abnormal reduction in salivary flow. Considering the stimulated saliva, rates below 0.7 ml/min are a signal of reduced flow (51, 52). The mean stimulated salivary flow found in this cluster was 0.7 mL/min, that is, some of the individuals presented low saliva flow rate. When it comes to obesity, the decrease in salivary flow can be caused by different mechanisms: the use of drugs for the treatment of comorbidities (hypoglycemic agents, antihypertensive drugs, and antidepressants) that can induce hyposalivation (53); other hypothesis is the fact that pro-inflammatory cytokines derived from adipocytes and macrophages accumulated in adipose tissue can negatively affect the function of the salivary glands due to mild chronic inflammation (54). In the present study, the commonly used drugs reported by the participants were antihypertensives, antidiabetic, antidepressants, and sedatives, and, according to Villa et al. (52), these are the main drugs reported as causing xerostomia/hyposalivation. In addition, the use of multiple drugs (polypharmacy) can promote pharmacodynamic effects and pharmacokinetic drug interactions, increasing the xerogenic potential of drugs (52).

In individuals with excess weight, the decreased salivary flow, pH, and buffer capacity may represent a major risk of oral health problems due to homeostatic imbalances. The impairment in salivary buffer capacity may lead to a reduced protection of the teeth, which are more susceptible to the development of dental caries and halitosis (1). Although the salivary pH found in this sample of individuals within the normal range of 6.8–7.5 (55, 56), the mean pH found in the analysis of buffer capacity may be considered low (57). Considering these findings, a multidisciplinary guidance is a paramount for individuals with morbid obesity to improve their oral and general health conditions.

This is the first study that includes a large sample of individuals with morbid obesity and has many implications for practice and further research. The study pointed out that even when examining a group of individuals with similar socioeconomic level and anthropometric status, this sample of individuals with morbid obesity showed heterogeneous groups (clusters) with different oral and systemic health characteristics and needs. Many of the causes of unsuccessful bariatric surgery are due to poor dietary behavior and compulsions to eat palatable foods that were present before surgery, hence the importance of recognizing the different profiles and planning the target treatments. Indeed, the cross-sectional nature of the findings and the use of a brief dietary questionnaire prevent cause-and-effect conclusions, representing the major limitation of the study, and future prospective studies should explore how diabetes associated to morbid obesity, in addition to insulin and hypoglycemic drugs intake, are related to hyposalivation, xerostomia and nutritional impairments.

CONCLUSION

Three clusters of individuals were identified, named "Young" (Cluster 1), "Diabetic individual" (Cluster 2), and "Poor periodontal health" (Cluster 3). Cluster 1 and 2 were the groups that showed higher demand for nutritional and dietetic counseling, because of the poorer eating behavior and higher serum glucose levels, respectively. On the other hand, Cluster 2 and 3 showed the higher demand for oral rehabilitation and dental treatment because of the loss of teeth and worse periodontal condition showed, respectively, besides the need for dietetic counseling. As oral and nutritional health-related aspects may vary given the multifactorial nature of these aspects, the identification of patterns and special needs is of clinical importance to adjust or change unhealthy habits and better assist this condition.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethical Research Committee of the Federal University of São Paulo (Protocol No. 1201/2017). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MM, MG, EL, and PC participated in the study design and conception of the study. MM, SC-J, and EP participated in data and sample collections and data curation. PC and IR supervised the data and sample collection. PC performed the statistical analysis. MM, MG, EL, and PC wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

FUNDING

This work was supported by the State of São Paulo Research Foundation (FAPESP, SP, Brazil, grants number 2016/10940-9 and 2017/26400-6). The funding source was not involved in the data collection, analysis, and interpretation; neither in the manuscript writing and in the decision to submit it for publication. We would like to thank FCT - Foundation for Science of Portugal, for the research contract CEECIND/04397/2017 to EL.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 15 January 2021
doi: 10.3389/fnut.2020.599737

Swallowing and Liking of Vegetable-Enriched Bread Compared With Commercial Breads as Evaluated by Older Adults

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Characteristics of food that influence liking and ease-of-chewing and swallowing are not well-understood. Reformulation of bread to improve nutrient density may improve liking, ease-of-chewing and swallowing which could improve dietary intake particularly with aging. The study aimed to compare objectively and subjectively four breads of increasing nutrient density: $1 white (WB) and wheatmeal (WMB) commercial breads and two in-house formulations of vegetable-enriched breads (VB75 or VB100) which incorporated drum-dried pumpkin and sweet corn flours for physical, sensory and ease-of-chewing and swallowing properties. Each bread underwent instrumental texture analysis. The commercial and vegetable-enriched breads were not different by hardness or springiness but the vegetable breads were up to 25% less cohesive, less gummy and less chewy than the commercial breads. Questionnaires and Likert scale (150 mm) responses were completed by 50 physically active volunteers aged 50+ years. Overall liking of the VB75 and VB100 was rated 40% higher than the white and wheatmeal breads. Vegetable-enriched breads were rated as almost 50% easier to chew (mean ± SD; WB 70.53 ± 39.46 mm, WMB 77.68 ± 33.13 mm, VB75 104.78 ± 30.69 mm, VB100 107.58 ± 24.90 mm) and swallow (WB 70.29 ± 37.98 mm, WMB 77.53 ± 34.88 mm, VB75 104.63 ± 28.25 mm, VB100 104.90 ± 25.54 mm). Vegetable-enriched breads compared to white and wheatmeal breads were instrumentally and subjectively less gummy, cohesive and chewy than commercial breads and have the potential to both improve nutrition and "ease of swallowing" in older people. New areas of research should explore other underutilized vegetables for bread enrichment and their ability to aid swallowing and improve nutrition status.

Keywords: older adult, bread, swallowing, sensory evaluation, crumb texture

OPEN ACCESS

Edited by:
Ana Carolina Mosca, University of Udine, Italy

Reviewed by:
Wendy J. Dahl, University of Florida, United States
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *28 August 2020*
Accepted: *07 December 2020*
Published: *15 January 2021*

Citation:
Amoah I, Cairncross C and Rush E (2021) Swallowing and Liking of Vegetable-Enriched Bread Compared With Commercial Breads as Evaluated by Older Adults. Front. Nutr. 7:599737. doi: 10.3389/fnut.2020.599737

INTRODUCTION

Bread, a staple food in New Zealand (1), contributes 11% of total daily energy intake and older people are more likely to choose wholegrain bread (60%) than younger people (1). Therefore, the reformulation of bread to improve its nutrition for older people could be attractive to breadmakers and consumers of bread.

In New Zealand, the yearly sales of bread increased between the year to 19/06/2016 and the year to 18/06/2017 by 3.5% (from $462,491,400 to $479,077,700) (2). In the same trading period, non-white bread and specialty bread also increased in sales value by 3.3% (from $233,008,800 to $241,028,800) and 6.6% (from $67,855,300 to $72,670,200) respectively (2). In addition to the supply of energy and the macronutrients carbohydrate, protein and fat, breads may have health effects and can be called "functional breads" (3). Drum-dried pumpkin and sweet corn powders are two potential functional ingredients that could be utilized in bread formulation. Bioactive compounds including carotenoids (4) and essential micronutrients such as potassium are present in pumpkin (5) whilst sweet corn is a source of dietary fiber (6). Despite the potential benefits associated with functional bread consumption, the incorporation of functional ingredients in bread will impact on its physical properties including the textural properties of bread crumb and sensory attributes (7). Thus, there is the need to ensure a balance between sensory attributes and *nutritional* properties.

Particularly for older people, difficulties associated with chewing and swallowing *may* affect the ability to ingest certain foods including bread (8, 9) which may result in poor outcomes in their nutrition and health status (10). Many older people have discretionary funds which potentially increase their ability to buy palatable and nourishing bread which is easy to swallow. There is therefore the need to evaluate the physical properties, sensory attributes including "ease of swallowing" and demand of breads with the target market of older consumers.

The objective of this study was to evaluate the physical, sensory and swallowing attributes of two vegetable-enriched breads compared with controls, two commercially produced breads $1 white and wheatmeal breads, with 50 older physically active adults aged 50+. The hypotheses were that:

a. The vegetable-enriched breads will have softer crumb texture than the control breads possibly due to their fiber/pectin composition that has good moisture keeping properties.
b. The higher pumpkin flour content vegetable-enriched bread will be easier to swallow than the lower pumpkin flour content vegetable-enriched bread, and both will be easier to swallow than the control commercial breads.
c. The vegetable-enriched breads will be more liked and older adult consumers would be willing to eat them at home *possibly due to its potential softer crumb property*.

DESIGN AND METHODS

This experimental study involved the formulation with eight wholesome ingredients, including drum-dried pumpkin and sweet corn vegetable flour, of two vegetable–enriched breads to meet the Nothing Else™ criteria (11). The physical and subjective (human participant) measures were compared to controls of $1 commercial white and wheatmeal breads.

The list of ingredients used for the vegetable bread formulation and places sourced is indicated below: strong white flour (Champion, Auckland), wholemeal flour (Champion, Auckland), whole flaxseed (Ceres Organic, Auckland), sprouted red wheat flour (Huckleberry, Auckland), pumpkin powder (Cedenco, Gisborne), sweet corn powder (Cedenco, Gisborne), yeast (Bakels, Auckland) and salt (Cerebos Skellerup, Auckland). In descending order by weight the ingredients were: strong white flour, wholemeal flour, *whole* flaxseed, sprouted wheat flour, pumpkin flour, sweet corn flour, fresh yeast and salt to produce two vegetable-enriched breads that differed only in the proportion of pumpkin powder; VB100 contained 25% more pumpkin powder (dry mix) than the VB75. The indirect method of bread-making was used for the bread development. A pre-ferment consisting of 225 g wholemeal flour, 150 g water, and 0.5 g instant yeast was developed for 2 min to tight dough consistency. The mixture was left covered for 12 h at 20°C to allow for fermentation. A 150 g portion of *whole flaxseed* was soaked in 180 g boiled water for 2 h at room temperature before placing in *a* chiller for 10 h. The final dough was prepared by mixing 450 g strong wheat flour, sprouted red wheat flour (115 g), pumpkin powder (75 g), sweet corn powder (20 g), salt (15 g), instant yeast (15 g) and water (600 g) for 8 min. The dough was developed for 6 min. The soaked *whole flaxseed* was added to the final mixture and mixed until incorporated. The dough was allowed to undergo bulk fermentation for one and half hours. The fermented dough was cut and shaped into logs and placed in tins and proved for an hour. The tins of dough were placed in a steam oven and baked at 215°C for 35 min. After baking, the VB75 breads were allowed to cool and packaged in transparent rubber packs. The same procedure was repeated for the VB100 breads except for the amount of pumpkin flour that was scaled up to 100 g. The recipe formulation for the vegetable breads used for the study is presented in **Table 1**. *The list of ingredients for the white bread included, in descending order by weight, wheat flour, water, baker's yeast, iodised salt, canola oil, acidity regulator (263), soy flour, emulsifier (481, 472e) and vitamin (folic acid). The wheatmeal bread contained wheat flour, water, wheatmeal flour, baker's yeast, vinegar, iodised salt, wheat gluten, acidity regulator (263), roasted barley malt flour, canola oil, soy flour, emulsifiers (481, 472e), and vitamin (folic acid).*

Nutritional information about the commercial breads was obtained from the nutrition information panel on the pack, for VB75 by proximate analysis at Asurequality, an Internationally Accredited New Zealand laboratory and for VB100 determined from the recipe and measured moisture loss using the software programme Foodworks 10 (Xyris, Brisbane) and the NZ food composition database (12) (**Table 2**). *The choice of the cheapest (NZ$1) commercial breads was premised on the fact that it is the most affordable bread in Countdown supermarket (50% of market share) in New Zealand.*

Physical Analyses: Texture Analysis

Texture profile analysis (TPA) measures of hardness, chewiness, cohesiveness, springiness, and resilience were determined using a (TA.XT.plus texture analyser, Stable Microsystems, Surrey, UK) with a 5 kg load cell. Crumb slices of 11.50 mm were 75% compressed. Parameters used include a pre-test speed of 5.00 mm/s, test speed of 1.00 mm/s, post-test speed of 5.00 mm/s, target mode-strain, time of 5.00 s and trigger force of 0.010 N. The

TABLE 1 | Recipe formulation for bread development.

Ingredient	VB75	VB100
Water	930 g	930 g
White strong flour	450 g	450 g
Wholemeal wheat flour	225 g	225 g
Flaxseed	150 g	150 g
Sprouted red wheat flour	115 g	115 g
Pumpkin powder	75 g	100 g
Sweetcorn powder	20 g	20 g
Instant yeast	5.5 g	5.5 g
Salt	15 g	15 g

VB75-Bread enriched with 75 g pumpkin flour and VB100-Bread enriched with 100 g pumpkin flour.

TABLE 2 | Proximate composition of breads.

Component	*WB	*WMB	†VB75	††VB100
Moisture (%)	36.57	38.19	39.11	46
Protein (g/100 g)	8.5	8.8	*NA*	6.5
Dietary fiber (g/100 g)	2.7	4.6	7.2	6.5
Insoluble fiber (%)	*NA*	*NA*	5.5	*NA*
Soluble fiber (%)	*NA*	*NA*	1.7	*NA*
Fat (g/100 g)	1.6	1.7	*NA*	*NA*
Carbohydrate (g/100 g)	46.7	43.1	*NA*	36
Sodium (mg/100 g)	392	398	380	380
Potassium (mg/100 g)	*NA*	*NA*	300	277
Energy (kJ/100 g)	1020	982	*NA*	889
β-carotene (μg/100 g)	*NA*	*NA*	236.78	*NA*

**As reported on the nutrition information panel. †Analysis by Asurequality, an Internationally Accredited New Zealand laboratory. ††Derived from recipe with the New Zealand Food Composition Tables (12). NA, not available; WB, white bread; WM, wheatmeal bread; VB75 and VB100, bread with 75 g and 100 g pumpkin substitution.*

resulting peak force was measured in grams. A minimum of five replicates from each of the sliced breads were averaged.

Participants

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the Auckland University of Technology Ethics Committee (AUTEC) (New Zealand, 18/22).

The number of participants required was calculated based on 108 previous studies of a wide range of food products including bread (13). *When the meaningful difference over a 150 mm Likert scale was set at 20%, with an α of 0.05 and β of 0.10 the number of participants required was 29.*

Participants recruited were older (50+ years) and physically active people who were registered members of the never2old group (an exercise programme), and regularly attend the Sport and Fitness Center at the Auckland University of Technology, North Shore campus, Auckland, New Zealand. The participants were advised about this study by the leaders of the never2old programme and the advertisement was posted on the notice boards in the fitness center. The researcher gave a brief presentation to potential participants prior to a fitness session and information sheets were distributed *and the procedure explained*. Participants who responded to the invitation were recruited in a chronological manner. Participants were excluded if they were receiving drugs that would affect taste (e.g., chemotherapy) or were gluten intolerant or allergic to any of the ingredients. Inclusion criteria were: aged more than 50 years, consumed bread at least once a week, and had no known allergy or intolerance to gluten. After a one-on-one opportunity was provided for participants to ask any questions, an appointment was made for the testing.

Fifty untrained participants (31 female, 19 male) consented to take part. One participant self-identified as Asian and the remainder as European. Twenty-six were aged 70–79 years (52%), twelve 80–89 years (24%), ten aged between 65–69 years (20%), and two were 50–59 years (4%).

Liking, Acceptability, and Swallowing

The widely-used questionnaire for liking and acceptability was as proscribed by Lawless and Heymann (14). *Briefly the liking of each bread sample in relation to the sensory attributes (color, aroma, taste, texture, mouthfeel, overall liking, and willingness to eat at home) was rated on seven 150 mm unstructured visual analog scales with anchor points of extremely dislike on the left and extremely like on the right. Similarly five 150 mm scales were used to estimate "ease of swallowing" evaluation (extremely difficult to extremely easy) followed the sequence of the passage of food from the lips to the throat (ease of biting and getting into the mouth, ease of chew, ease of swallow, ease of throat movement, less stickiness in throat) and the number of chews before swallowing was counted by the participant. The swallowing questions were pre-tested for face validity and readability by colleagues.*

Participants attended the sensory evaluation sessions in a sensory room at the fitness center. *The procedure and the questionnaire was explained and demonstrated to the participants and opportunities to ask questions provided throughout. Participants then were asked to sign a consent form.* Each participant was seated at a table so that they could not see other participants. *On the table* were two slices each of each of the four different breads (one slice with crust and a second de-crusted slice of 11.50 mm square). *The sliced bread with intact crust weighed approximately 50 g.* The bread was served in an unrandomised (first 29 participants) and randomized (next 21 participants) order to the consumers on white plates identified with random three-digit numbers. For logistical reasons each portion of bread had been stored sealed and frozen and was allowed to defrost, sealed for a 1-h period. *Each bread was identified by a unique number.* Water was used to rinse the mouth between breads to minimize any residual effect between *breads. One questionnaire was provided* for each bread.

All the analyses were *undertaken with the* Statistical Package for Social Sciences (SPSS) version 24.0 software (IBM, New York). The results on physical properties and ease of swallowing of bread were subjected to one-way analysis of variance (ANOVA) *with bread as the grouping variable.* Means and 95% confidence interval *of differences in means are* reported. *Post hoc* Tukey's test was used to compare the mean values and establish significance differences at $p < 0.05$. *Although the initial unrandomisation order of presentation of the breads to the*

TABLE 3 | Physical and textural attributes of the four breads.

Characteristics	N	Bread samples				
		WB	WMB	VB75	VB100	*P*-value
Physical, textural attributes						
Loaf weight (g)	4			420.0 ± 1.6[a]	421.8 ± 2.5[a]	0.556
Baking loss (%)	4			10.4 ± 0.3[a]	10.1 ± 0.5[a]	0.556
Loaf volume (ml)	4			1,027.7 ± 25.1[a]	1,047.3 ± 11.9[a]	0.381
Specific loaf volume (mL/g)	4			2.5 ± 0.1[a]	2.5 ± 0.0[a]	0.498
Loaf crust color						
L*	7	57.1 ± 6.5[b]	53.26 ± 1.7[b]	33.2 ± 1.0[a]	35.56 ± 4.1[a]	<0.0001
a*	7	17.5 ± 1.3[b]	17.4 ± 1.1[b]	14.3 ± 0.9[a]	15.4 ± 0.7[a]	<0.0001
b*	7	35.5 ± 2.9[b]	33.0 ± 1.3[b]	19.2 ± 1.2[a]	22.1 ± 2.6[a]	<0.0001
Loaf crumb color						
L*	10	83.7 ± 2.8[c]	77.8 ± 2.5[b]	55.0 ± 4.5[a]	55.4 ± 3.6[a]	<0.0001
a*	10	−0.1 ± 0.2[a]	2.0 ± 0.6[b]	3.2 ± 0.8[c]	4.3 ± 1.4[d]	<0.0001
b*	10	10.3 ± 1.0[a]	15.3 ± 1.8[b]	36.1 ± 2.5[c]	40.0 ± 1.8[d]	<0.0001
Hardness (g)	5–9	8.49 ± 1.74[a]	8.51 ± 1.00[a]	8.68 ± 2.23[a]	10.06 ± 1.09[a]	0.131
Resilience (%)	5–9	28.78 ± 3.14[b]	33.41 ± 3.22[c]	27.06 ± 3.23[b]	20.58 ± 2.65[a]	<0.0001
Cohesion	5–9	0.74 ± 0.05[c]	0.80 ± 0.04[c]	0.60 ± 0.06[b]	0.52 ± 0.02[a]	<0.0001
Springiness (%)	5–9	84.74 ± 17.87[a]	91.51 ± 1.84[a]	87.55 ± 4.46[a]	82.36 ± 5.62[a]	0.170
Gumminess	5–9	6.21 ± 1.14[a,b]	6.80 ± 0.80[b]	5.19 ± 1.36[a]	5.23 ± 0.51[a]	0.006
Chewiness	5–9	5.41 ± 1.81[a,b]	6.23 ± 0.79[b]	4.59 ± 1.33[a]	4.30 ± 0.43[a]	0.007

Data is expressed as mean ± standard deviation. Values with different superscript in a row are significantly different ($p < 0.05$). WB, white bread; WMB, wheatmeal bread, VB75 and VB100, breads with 75 g and 100 g pumpkin substitution. Means with different superscripts in a column are significantly different ($p < 0.05$). N= Number of replicates. L indicates the lightness value, a* indicates the degree of redness, and b* indicates the degree of yellowness. A higher the L*, a*, and b* value means a higher degree of lightness, redness, and yellowness of the bread, respectively. A higher hardness, resilience, cohesion, gumminess, and chewiness value indicates a higher degree of bread crumb hardness, resilient, cohesion, springinessy, gumminess, and chewiness of the bread crumb, respectively, following the double compression measurements of using the texture analyser probe.*

participants was a limitation, there were no significant differences between the attributes recorded by participants by randomized or not ($p > 0.05$) and the data was pooled for the final analysis. Sigma plot® software was used to visually establish the relationship between the objective and subjective perceptions associated with ease of swallowing.

RESULTS

Loaf weight, baking loss, loaf volume and specific volume of the VB75 and VB100 breads were not significantly different (**Table 3**). The VB75 and VB100 breads had substantially darker (>40% darker) crusts than the WB and WMB. The lightness, redness, and yellowness colors of the crusts of the VB75 and VB100 breads were not different.

Physical measures of hardness and springiness among the four breads were not significantly different. The WMB was the most resilient to compression and had higher cohesion than the two vegetable-enriched breads but not the WB (**Table 3**). Resilience and cohesion of VB75 were higher than VB100 but springiness, gumminess and chewiness were not different between the two vegetable-enriched breads. Objective chewiness was higher for the WMB compared with both the VB75 (1.64 units, 95% CI 0.42, 3.25 $p = 0.043$) and VB100 (1.93 units, 95% CI 0.49, 3.37, $p = 0.006$) but not the WB.

Evaluated by the participants, the VB75 and VB100 breads were liked almost twice as much as the WB and WMB for all the sensory attributes assessed. The participants also stated that they were willing to eat the VB75 and VB100 breads at home (**Table 4**). There were no differences between the WMB and the WB for the liking attributes except for the color *of* the WMB *which was liked more* than the WB. Both the VB75 and VB100 recorded scores almost twice those of the WB and WMB for willingness to eat at home (**Table 4**).

WB was perceived as more difficult to bite and get into the mouth, chew, swallow, move through the throat compared with the other breads (**Table 4**) and also that it stuck in the throat more during swallowing. The VB100 was perceived as the easiest to chew and swallow and moved more easily through the throat with less throat stickiness. The swallowing attributes of VB75 bread were, however, not significantly different from the VB100 in terms of ease of bite and getting into the mouth ($p = 0.99$), ease of chew ($p = 0.97$), ease of swallow ($p = 1.00$), ease of throat movement ($p = 0.99$) and less stickiness in throat ($p = 0.96$). The overall liking of all the breads was strongly correlated with the ease of swallowing: VB75, $r = 0.597$, 95% (0.3820.751); VB100, $r = 0.665$, 95% (0.474–0.796); WB, $r = 0.422$, 95% (0.163–0.627); and WMB, $r = 0.475$, 95% (0.227–0.665).

Participants reported the two commercial breads as less easy to chew, to get in the mouth and swallow than the vegetable-enriched breads (**Figure 1** and **Table 4**) which is in the

TABLE 4 | Sensory liking and swallowing perceptions of the four breads by participants ($n = 50$).

Characteristics	Bread samples				
	WB	WMB	VB75	VB100	*P*-value
Sensory attributes: liking (mm/150 mm)					
Color	54.24 ± 37.23[a]	80.65 ± 34.42[b]	85.74 ± 33.42[b]	87.54 ± 37.66[b]	<0.0001
Aroma	73.12 ± 31.06[a]	76.16 ± 27.49[a]	95.17 ± 27.30[b]	92.16 ± 34.12[b]	<0.0001
Taste	52.77 ± 31.09[a]	60.22 ± 29.27[a]	93.42 ± 30.00[b]	94.10 ± 34.27[b]	<0.0001
Texture	46.70 ± 30.81[a]	60.71 ± 30.93[a]	96.69 ± 28.78[b]	97.60 ± 31.89[b]	<0.0001
Mouthfeel	47.46 ± 30.26[a]	54.34 ± 27.57[a]	94.36 ± 27.57[b]	91.09 ± 36.44[b]	<0.0001
Overall liking	40.85 ± 31.59[a]	52.43 ± 32.80[a]	92.03 ± 34.24[b]	93.58 ± 35.52[b]	<0.0001
Willing to eat at home	30.19 ± 33.40[a]	47.64 ± 34.24[a]	84.67 ± 42.89[b]	86.47 ± 42.21[b]	<0.0001
Swallowing evaluation/150 mm					
Ease of biting and getting into the mouth	98.31 ± 31.79[a]	99.56 ± 26.53[a,b]	113.29 ± 28.32[b]	111.34 ± 26.47[a,b]	0.012
Ease of chew	70.53 ± 39.46[a]	77.68 ± 33.13[a]	104.78 ± 30.69[b]	107.58 ± 24.90[b]	<0.0001
Ease of swallow	70.29 ± 37.98[a]	77.53 ± 34.88[a]	104.63 ± 28.25[b]	104.90 ± 25.54[b]	<0.0001
Ease of throat movement	77.33 ± 39.75[a]	78.18 ± 34.78[a]	108.61 ± 27.53[b]	110.41 ± 23.03[b]	<0.0001
Less stickiness in throat	72.33 ± 45.45[a]	75.94 ± 39.64[a]	107.62 ± 33.46[b]	111.48 ± 29.18[b]	<0.0001
Number of chews before swallowing	19	21	18	19	

Data is expressed as mean ± standard deviation. Values with different superscript in a row are significantly different ($p < 0.05$). Scoring is on a Likert scale 0–150 mm with 0 mm as the least and 150 mm as the most acceptable. WB, white bread; WM, wheatmeal bread; VB75 and VB100, breads with 75 g and 100 g pumpkin substitution. Means with different superscripts in a column are significantly different ($p < 0.05$).

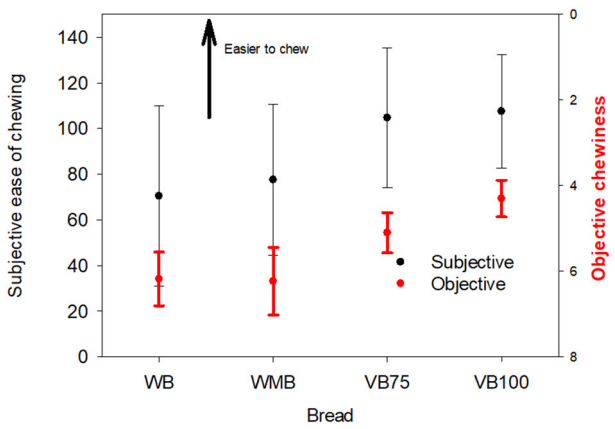

FIGURE 1 | Relationship between subject-rated ease of chewing and objective chewiness of bread samples. Means and 95% CI. WB, white bread; WM, wheatmeal bread; VB75 and VB100, bread with 75 g and 100 g pumpkin substitution.

same direction as objective measures which discriminated the commercial breads as chewier, more cohesive, and gummier than the vegetable breads.

DISCUSSION

Vegetable-enriched breads were successfully formulated and produced. This study has shown that older participants substantially preferred the taste of the VB75 and VB100 breads over the commercial breads and would be willing to eat the vegetable-enriched breads at home. They also subjectively ranked the vegetable-enriched breads as easier to chew and swallow than the commercial white and wheatmeal breads. This confirmed the hypotheses that the higher pumpkin flour concentration vegetable-enriched bread (VB100) will be easier to swallow than the lower pumpkin concentration vegetable-enriched bread (VB75), and both will be easier to swallow than the commercial control breads (WB and WMB). The objective measures of chewiness and cohesion were the only physical measures able to differentiate between the commercial breads and the vegetable-enriched breads in the same direction as the participants' perceived ease of chewing. A novel ease-of-swallowing "solid food" questionnaire was trialed, found to be understood by and acceptable to participants, and showed subjective discrimination of the ease of stages of ingestion among breads that the objective measures did not.

Explanations for the easier chewing and swallowing of the vegetable-enriched breads related to an easier formation and passage of the bread bolus. This may be attributed to pumpkin powder containing pectin (15) which is rich in hydrophilic fibers (16), increasing the bulk of the bread in the mouth and a potential increased saliva stimulation. Saliva promotes the formation of cohesive network between the bread bolus in the mouth, consequently aiding swallowing (17). *Soaking of unground flaxseed results in the formation of a viscous mucilage which has emulsifying properties* (18). *Additionally, flaxseed has a fat content of 42.2 g/100 g* (12). *Fats reduce the adhesiveness of the food bolus formed in the mouth and thus putatively, if well chewed, could partially contribute to the ease of swallowing of the bread bolus. Gluten from the wheat flour, provides viscoelasticity*

to the dough and helps with the retention of carbon dioxide gas produced after fermentation (19, 20). *The addition of the vegetable powders to the wheat flour dilutes the gluten which may have the effect of softening the bread making it easier to chew and swallow. In addition, flaxseed by mass is ∼25% fiber, 70% insoluble and contributed substantially (30%) to the total fiber in the bread* (12). *It is therefore likely that flaxseed is the ingredient that may have augmented many of the outcomes, and in particular texture.*

Increased salivation *may be* stimulated by color of bread (21) *and can impact* on consumers' choices. Bread with white crumb is perceived to be *less healthy*, particularly amongst older people, as indicated anecdotally by the participants. The yellow color of the VB probably created an impression of healthiness in the minds of the participants, consequently resulting in the higher liking of the VB. Kraus (22) reported that drivers for consumers' liking of food are dependent on healthiness and naturalness.

The vegetable-enriched breads were more liked for their taste compared to the commercial WB and WMB breads. A plausible reason could be the action of saliva as a medium for the dilution of taste compounds including sugar and salt (23) from the vegetable-enriched breads. The diluted compounds are subsequently conveyed to taste receptors on the surface of the tongue (24, 25) and are perceived to be appealing by the participants.

An explanation for the liking of the aroma of the vegetable-enriched breads could be in relation to the role of saliva. Mosca and Chen (24) postulated that saliva increases the availability of aroma compounds from food *as the* food *is* broken into smaller particle sizes during chewing. The released aroma compounds attach themselves to receptors in the mouth while some diffuse into cavities of the nose leading to flavor perception which in turn results in increased salivation (26). Aroma released from bread also impacts on the release of saliva. Studies on the flavor volatiles available in sweet corn revealed the presence of aroma compounds including dimethyl sulfide, 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline (27). Interestingly, 2-acetyl-1-pyrroline, which is an essential flavor compound produced from Maillard reaction in sweet corn, is noted for its appealing flavor in bread (28). 2-acetyl-2-thiazoline, on the other hand, is found to generate a roasty popcorn-like favor in bread (28). Other compounds including hydrogen sulfide, methanethiol, acetaldehyde, ethanol, ethanethiol, dimethyl sulfide impact the aroma of thermally processed sweet corn (29). The presence of these compounds in addition to the Maillard reaction that takes place during the baking process possibly resulted in the generation of appealing aromatic compounds in the vegetable-enriched breads. This could be attributed to the degradation and modification of the cell walls of the vegetable ingredients which may result in an aroma favorable to the consumers (30). A strong positive association between the liking of food and its aroma composition has been reported (31) thus the higher liking score recorded for the vegetable-enriched breads by the participants may be justified. Additionally, the pre-fermentation of the wholemeal flour for 12 h using yeast possibly improved the textural properties and promoted the release of certain volatile and aromatic compounds in the vegetable-enriched breads.

It is also worth highlighting that during bread chewing, the texture of the bread matrix impacts on the release of aroma compounds (32). *In the present study, participants had a favorable perception of the crumb texture of the vegetable-enriched breads leading to an appealing mouthfeel. Consequently, salivation of the bread bolus increased, resulting in the ease of bread swallowing.* The vegetable-enriched breads were reported to be easier to chew. Chewing is a mechanical process that stimulates the release of saliva (33) consequently promoting increased bread bolus lubrication (17). This possibly resulted in easier swallowing and movement of the bread bolus through the throat (24).

With aging the mass of the swallowing muscles declines (34) *and swallowing may be less efficient. Thus, for older people*, the vegetable-enriched Nothing Else™ breads, if consumed, could be favorable for their overall nutritional intake.

The older participants subjectively found white bread more difficult to chew and swallow though there was no difference between its crumb hardness and that of the vegetable-enriched breads as evaluated objectively. This was expected, as white bread is formulated from refined white flour, poor in fiber and contains emulsifiers which improves bread crumb textural attributes (35, 36). Consequently, after bread chewing, bolus swallowing may get impaired as it clogs in the throat, and this was confirmed anecdotally by the participants. The commercial sold white and wheatmeal breads contained emulsifiers 481 (sodium oleyl lactylate, sodium stearoyl lactylate, and sodium lactylate) and 472e (diacetyltartaric and fatty acid esters of glycerol) (37) to improve the bread crumb textural properties and to cause the bread to feel softer in the plastic bag and therefore appear fresher (36). This likely contributed to the lower hardness of the white breads when measured with the texture analyser, as the use of emulsifiers has been reported to improve the textural properties of bread (35, 36). The use of food additives including hydrocolloids to enhance the properties of bread, especially in commercially sold bread, is a common and accepted practice. However, particularly amongst the health-conscious older population, consumers are avoiding food products with food additive enhancement as they see them as "unnatural" (38).

There is potentially an adverse effect of the higher moisture content in the vegetable-enriched bread VB100 as higher water activity leads to early spoilage by microbiological organisms, especially mold (39–41). However, in a review paper recently published (42), we posited that functional breads tend to have a longer shelf life than white breads due to antimicrobial and antioxidant bioactivity compounds present in the functional ingredients which impair the growth rate of mold and the oxidation of lipids and fats in the bread matrix.

STRENGTHS AND LIMITATIONS

This analysis was limited by the relatively small number of breads to compare (4) *and the convenience sample of healthy older adults enrolled in a fitness programme. The participants were not asked if they had any difficulties swallowing food or*

if they required water to help them swallow. In addition, the unavoidable lack of blinding to, for example, the color and appearance of the breads may have created bias in the assessment of ease of swallowing. The initial lack of randomisation of order of presentation of the breads may also have created bias but there appeared to be no difference between the randomized versus unrandomised results so they were pooled. Additional experiments and measurements would provide more information and helped identify ingredients responsible for the characteristics but also would increase participant burden, reduce compliance and lengthen the swallowing questionnaire. More food chemistry analyses of the breads would provide the information missing in ***Table 2****. Future comparison of swallowing characteristics of foods reformulated for a better nutritional profile and easier swallowing would aid both food manufacturers, marketeers and consumers.*

CONCLUSION

The enrichment of bread with pumpkin and sweet corn and the pre-ferment dough preparation was apparently associated with overall liking and improved ease of chewing and swallowing. Future interdisciplinary research should focus on understanding the microstructure of foods and subjective ease of swallowing. New areas of research should explore other underutilized vegetables for bread enrichment and their ability to aid swallowing and improve nutrition status, and the utility of a subjective swallowability index for foods for consumers.

DATA AVAILABILITY STATEMENT

The anonymised raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Auckland University of Technology Ethics Committee (AUTEC) (New Zealand, 18/22). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

IA formulated the research question, designed the study, carried it out, analyzed the data and prepared the first draft. CC provided logistic support and supervision. ER obtained the funding for IA, oversaw the research process from conception, analyzed the data and provided logistic support and supervision. All authors provided critical revision of the article.

FUNDING

IA was supported by a Ph.D. scholarship from the Riddet Institute, a New Zealand Center of Research Excellence, funded by the Tertiary Education Commission. The research was supported by funding from the Auckland University of Technology and undertaken at the Auckland University of Technology.

ACKNOWLEDGMENTS

The authors thank the participants from the Never2old group from the Sport and Fitness Center at the Auckland University of Technology. We are grateful for the culinary expertise of Arno Sturny in the formulation and preparation of the vegetable breads. AUT food network members are thanked for their support.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

REVIEW
published: 28 January 2021
doi: 10.3389/fnut.2020.612735

The Relationship Between Salivary Redox, Diet, and Food Flavor Perception

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The mouth is the gateway for entrance of food and microorganisms into the organism. The oral cavity is bathed by saliva, which is thus the first fluid that food and microorganisms will face after their entrance. As a result, saliva plays different functions, including lubrication, predigestion, protection, detoxification, and even transport of taste compounds to chemoreceptors located in the taste buds. To ensure its function of protection, saliva contains reactive harmful compounds such as reactive oxygen species that are controlled and neutralized by the antioxidant activity of saliva. Several antioxidant molecules control the production of molecules such as reactive oxygen compounds, neutralize them and/or repair the damage they have caused. Therefore, a balance between reactive oxidant species and antioxidant compounds exists. At the same time, food can also contain antioxidant compounds, which can participate in the equilibrium of this balance. Numerous studies have investigated the effects of different food components on the antioxidant capacity of saliva that correspond to the ability of saliva to neutralize reactive oxygen species. Contradictory results have sometimes been obtained. Moreover, some antioxidant compounds are also cofactors of enzymatic reactions that affect flavor compounds. Recent studies have considered the salivary antioxidant capacity to explain the release of flavor compounds *ex vivo* or *in vivo*. This article aims to review the effect of food on the antioxidant capacity of saliva and the impact of salivary antioxidant capacity on flavor perception after a brief presentation of the different molecules involved.

Keywords: saliva, antioxidant capacity, diet, flavor, redox, perception, antioxidant, salivary proteins

OPEN ACCESS

Edited by:
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University of Évora, Portugal

Reviewed by:
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Francisco Amado,
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *30 September 2020*
Accepted: *31 December 2020*
Published: *28 January 2021*

Citation:
Schwartz M, Neiers F, Feron G and Canon F (2021) The Relationship Between Salivary Redox, Diet, and Food Flavor Perception. Front. Nutr. 7:612735. doi: 10.3389/fnut.2020.612735

INTRODUCTION

This review reports the relationships that have been established in the literature between the salivary antioxidant capacity, the diet and the perception of food flavor. As several reviews have already approached the relationship between salivary antioxidant capacity and pathologies (1, 2), this aspect will only be briefly discussed in this review.

Saliva is a complex biological fluid that plays an important role in bodily protection and consequently in health (3). In addition to protecting the oral cavity against microorganisms and abrasion by food particles (4, 5), saliva ensures several functions in food perception (6). Indeed, saliva ensures the transport of tastants and trophic factors to the taste buds (7), and thus allows the detection of food compounds including energetic and toxic compounds by the taste receptors. Protection of the oral cavity involves the secretion of numerous salivary proteins such

as immunoglobulins or enzymes that regulate the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS and RNS react with biomolecules including proteins, lipids and nucleic acids. Consequently, these species are toxic toward epithelial cells and the microorganisms living on the oral tissues. Thus, the regulation of these reactive species through the production of antioxidant compounds is essential for the organism. Food also contains oxidizing, reducing and antioxidizing compounds that can affect, at least temporally, the antioxidant status of the oral cavity. Moreover, the antioxidant capacity of saliva has recently been suggested to modulate the metabolization of flavor compounds (8, 9) and thus impact their release (8–11) and effect on their perception (12). The first part of this review introduces the salivary redox status and the involved species, the second section reports on the relationship between salivary antioxidant capacity and physiologic status capacity and finally we present the links between salivary redox, diet and food perception.

SALIVARY REDOX STATUS

Reactive Oxygen Species

Reactive Oxygen Species Origins

Molecular dioxygen is essential for cellular respiration but is toxic. Even if this toxicity is low, it can lead to the formation of much more reactive species and consequently toxic species called reactive oxygen species (ROS). ROS can be generated by many physiological processes such as cellular respiration but is also used as a defense body during an immune response. ROS include oxygen radical forms that are very reactive despite a very short lifespan (less than a millisecond). The effects of these radical forms mainly result from their reactions with lipids, nucleic acids (13) and proteins (14). In the mouth, ROS are generated in the oral epithelium and directly in the saliva. ROS production in the oral cavity regulates the oral microbiota. Furthermore, ROS production is also physiologically limited to prevent many pathologies including inflammatory syndromes or even oral cancers such as oral leukoplakia (15). Many exogenous factors can lead to the deregulation of the redox balance by acting directly on the oral cavity, namely, in a non-exhaustive manner: the use of tobacco (16), certain pharmaceutical molecules, and many pro- or antioxidants present in the food naturally or artificially as certain additives. Interestingly, the deregulation of saliva redox balance is a good diagnostic indicator for many pathologies that are not only directly linked to the oral cavity (HIV, diabetes, renal dysfunction, etc.) (1).

Reactive Oxygen Species Types

There are many types of ROS. Without being exhaustive, those that will be important from the point of view of their consequences in terms of reactivity with biological molecules or generation of other ROS will be mentioned here.

Superoxide anion ($O_2{\cdot}^-$) is the precursor of many ROS. The superoxide anion is produced by the monoelectronic reduction of dioxygen naturally during cellular respiration but can also result from enzymatic production [e.g., xanthine oxidase in milk (17)]. Superoxide anion reacts with many endogenous (e.g., haemoproteins) or exogenous molecules, especially those contained in food (e.g., sulfites, thiols, and quinones) (18).

Hydrogen peroxide (H_2O_2) results from the dismutation of the superoxide anion. This dismutation can be catalyzed by superoxide dismutase, an enzyme found in human saliva. Hydrogen peroxide can also be produced and released in saliva by oral microbiota (19). Importantly, hydrogen peroxide can cross cell membranes and serve as a secondary messenger in many cellular processes (20). The diffusion capacity of hydrogen peroxide has a high impact because it is the source of one of the most reactive ROS: the hydroxyl radical (OH·). Superoxide anion also leads to the generation of the hydroxyl radical. This highly reactive ROS can react with many molecules although its high reactivity limits its diffusion. Two main reactions lead to its production: the Haber-Weiss reaction between superoxide anion and hydrogen peroxide (21) and the Fenton reaction between reduced iron (Fe^{2+}) and hydrogen peroxide (22).

Other ROS can be synthesized enzymatically, such as nitric oxide (•NO) by nitrite oxide synthase, which is produced in submaxillary glands (23), from arginine (24) or hypochlorous acid from chloride and hydrogen peroxide by myeloperoxidase, which is also present in saliva (see paragraph Elimination of ROS). Hydrogen peroxide is particularly toxic in its ability to form the hydroxyl radical by reacting directly with superoxide anion without requiring the Fenton reaction (25).

Regarding RNS, these are also reactive species derived from nitric oxide and superoxide that are produced by nitric oxide synthase 2 and NAPDH oxidase, respectively.

Salivary Antioxidant Capacity

Saliva contains many molecules. Among them, many have antioxidant capacity that limits ROS generation. The salivary oral antioxidant capacity results from a combination of different molecular mechanisms (see **Figure 1**). For some reactions, the antioxidant power of these molecules requires enzymatic activities. Recent proteomic studies (26–29) have identified numerous antioxidant proteins or enzymes in saliva (see **Table 1**), thus providing insights into its antioxidant capacity.

Elimination of ROS

To prevent the formation of the hydroxyl radical, specific enzymes were selected during evolution. Some enzymes remove superoxide anion thus preventing the Haber-Weiss reaction, while others detoxify hydrogen peroxide and thus prevent this same reaction but also the Fenton reaction. In addition, low-molecular weight molecules act directly by neutralizing ROS.

The main neutralizing enzymes are superoxide dismutases (SOD), which are metalloproteins that catalyze the dismutation of two $O_2^{\cdot-}$ molecules into H_2O_2 (30). These enzymes are the front line factors that directly remove superoxide anion (31). The formed hydrogen peroxide can then be eliminated by the catalase or peroxidase systems described in the following paragraph.

Catalase plays an important role because it allows NADPH-dependent dismutation of H_2O_2 into H_2O and O_2 (32). Historically, catalase activity measured in the saliva has been proposed to be mainly bacterial, with variability between healthy individuals and individuals with periodontitis (33). More

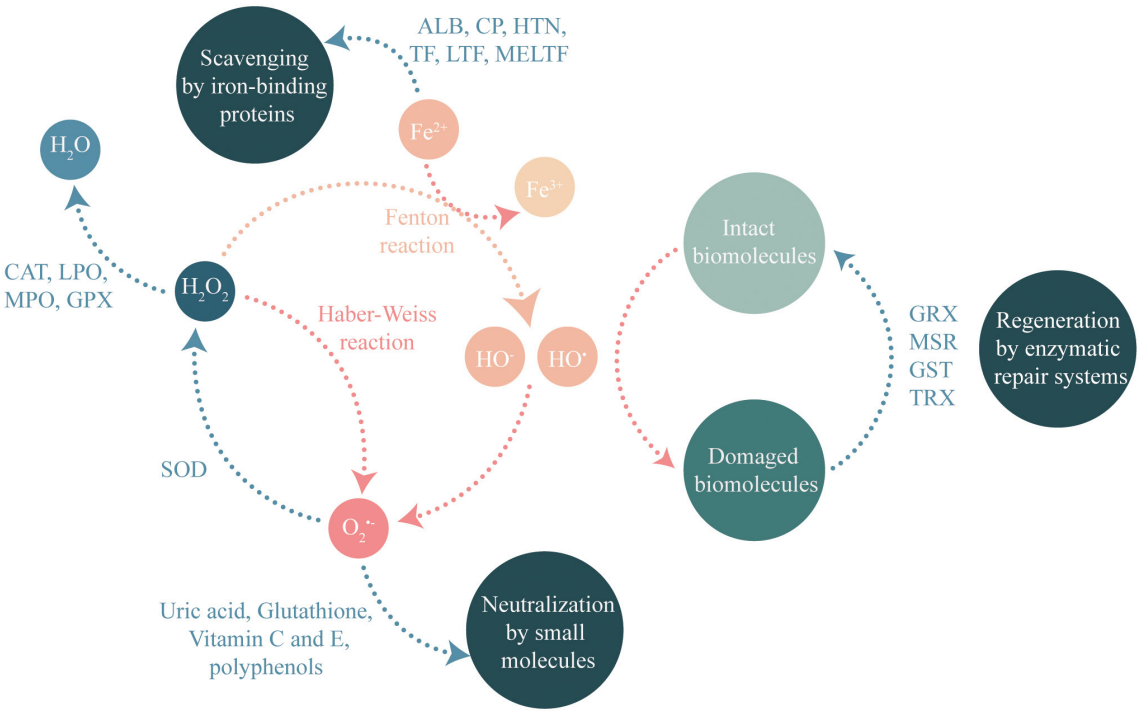

FIGURE 1 | Molecular mechanisms controlling ROS concentration and participating to antioxidant capacity of saliva.

recently, proteomic studies have shown that human catalase is also present in the saliva, thus indicating that salivary catalase activity has a dual human and bacterial origin (26). Catalase activity in the saliva is correlated with several diseases or habits. Salivary catalase activity is in increased in type I diabetics (34) but decreased in smokers (35). A second important family of enzymes involved in hydrogen peroxide detoxification are the peroxidases. Two peroxidases are present in the saliva: lactoperoxidase (also called salivary peroxidase) produced by the parotid and submandibular glands and myeloperoxidase contained in polymorphonuclear neutrophils (36). These enzymes, in addition to hydrogen peroxide reduction, also exhibit an antimicrobial potential through oxidation of the thiocyanate ion (36). The formed hypothiocyanite ion limits bacterial proliferation in the mouth by oxidizing thiol residues in essential microbial proteins (37) and can also inactivate human salivary detoxification proteins (38). Therefore, antioxidant systems appear essential in the maintenance of salivary redox balance.

In addition to enzymatic systems, low-molecular weight molecules also neutralize radical species. These molecules present diverse structures and functional groups (aromatic rings, hydroxyl groups). The main antioxidant molecule in human saliva is uric acid, which is responsible for nearly 70% of the antioxidant activity of saliva (39) with a concentration between 40 and 240 μM (39–42). The concentration of uric acid in the saliva correlates with its concentration in the plasma, suggesting that it comes from this fluid (43). Other small molecules are also involved in direct ROS scavenging including vitamin C (ascorbic acid) and vitamin E (tocopherols and tocotrienols) at cell membranes.

Maintenance of Salivary Redox Balance

Among the amino acid residues, cysteine and methionine are the most sensitive to oxidation. The oxidation of cysteine generates sulfenic acid that leads to the formation of a disulfide bridge after reaction with a second cysteine residue. In some cases, the oxidation of cysteine to sulfenic acid can lead to higher oxidation states, namely, sulfinic acid followed by sulfonic acid, which are more difficult to reverse. These oxidations cause the inactivation of thiol enzymes [e.g., cysteine proteases and salivary cystatins (44) or some salivary detoxification enzymes (38)], and can lead to the aggregation of salivary proteins (45). The reduction of thiols therefore enables to maintain the function of the salivary proteins containing thiols and additionally to absorb oxidation. Systems allowing thiol reduction (disulfide bridge or sulfenic acid) are therefore essential to maintain redox balance. At a concentration of ∼600 μM (46), the main salivary thiol-type antioxidant is glutathione (tripeptide γ-L-glutamyl-L-cysteinyl-glycine). Glutathione can act directly on ROS (hydrogen peroxide and chlorinated oxidants), but its action on oxidized molecules can also be catalyzed by glutathione-dependent enzymes present in the saliva. Indeed, glutathione is a cofactor that allows electron transfer to reduce oxidized species. The oxidation of glutathione causes the formation of glutathione disulfide, and regeneration is carried out by glutathione reductase with NADPH as an electron donor. As a cofactor, glutathione is used by numerous salivary enzymes such as:

TABLE 1 | List of enzymes identified in proteomic studies that have an antioxidant activity.

Process	Proteins	Genes	Accession numbers	Antioxidant functions	References
ROS scavenging	Catalase	CAT	P04040	H_2O_2 detoxification	(32)
	Lactoperoxidase	LPO	P22079	H_2O_2 detoxification, hypothiocyanous ion synthesis	(36)
	Myeloperoxidase	MPO	P05164	H_2O_2 detoxification, hypothiocyanous ion synthesis	(36)
	Peroxiredoxins, isoforms 1 to 6	PRDX1 to PRDX6	Q06830, P32119, P30048, Q13162, P30044, P30041	Peroxides detoxification	(53)
	Superoxide dismutases, isoforms 1 to 3	SOD1, SOD2, SOD3	P00441, P04179, P08294	Superoxide radical dismutation	(30)
Redox maintenance and GSH-dependent enzymes	Glutaredoxins, isoforms 1, 3, and 5	GLRX, GLRX3, GLRX5	P35754, O76003, Q86SX6	Reduction of glutathionylated proteins	(53)
	Glutathione peroxidases, isoforms 1, 3, and 4	GPX1, GPX3, GPX4	P07203, P22352, P36969	Glutathione-dependent peroxides detoxification	(53)
	Glutathione reductase	GSR	P00390	Glutathione regeneration	(53)
	Glutathione transferases, isoforms from classes alpha, pi, mu, theta, and omega	GSTA1, GSTA4, GSTP1, GSTM1, GSTM2, GSTM4, GSTT1, GSTT2, GSTO1	P08263, O15217, P09211, P09488, P28161, Q03013, P30711, P0CG29, P78417	Xenobiotic detoxification, glutathione-dependent peroxides detoxification, ascorbate regeneration	(157)
	Methionine sulfoxide reductase A	MSRA	Q9UJ68	Reduction of methionine-sulfoxide in proteins	(58)
	Protein disulfide-isomerases	PDIA3, PDIA4, PDIA6, P4HB	P30101, P13667, Q15084, P07237	Disulfide bond formation	(53)
	Sulfhydryl oxidase	QSOX1	O00391	Disulfide bond formation	(53)
	Thioredoxin reductase	TXNRD1	Q16881	Dithiol-disulfide exchange reactions	(53)
	Thioredoxins, isoforms 1 and 2	TXN, TXN2	P10599, Q99757	Dithiol-disulfide exchange reactions	(53)
Metal binding	Albumin	ALB	P02768	Cd, Co, Cu, Fe, Hg, Ni, Zn binding	(158)
	Ceruloplasmin	CP	P00450	Fe, Cu binding	(159)
	Histatins, isoforms 1 and 3	HTN1, HTN3	P15515, P15516	Cu, Fe binding	(61)
	Lactotransferrin	LTF	P02788	Cu, Fe, Mn, Zn binding	(160)
	Melanotransferrin	MELTF	P08582	Fe binding	(161)
	Serotransferrin	TF	P02787	Fe binding	(162)

- glutathione peroxidase, which is involved in the glutathione-dependent decomposition of peroxides (47),
- glutathione transferases involved in the detoxification of xenobiotics and the elimination of lipid peroxidation products such as 4-hydroxy-2-nonenal (48),
- glutaredoxin, which ensures the reduction of glutathionylated proteins (49) and vitamin C (50). High levels of glutaredoxin have been detected in calf saliva (51). Several proteomic studies on human saliva have identified members of the glutaredoxin family (26, 27, 52).

Thioredoxin, the main enzyme involved in disulfide bridge reduction is found in human saliva (26–29). The thioredoxin system is made of thioredoxin reductase, which reduces thioredoxin, and NADPH, which is the final electron donor (53). Thioredoxin is multifunctional. In addition to its role as a regulator of protein thiol functions, thioredoxin can also directly neutralize certain ROS such as hydroxyl radical or singlet oxygen (54, 55). The levels of thioredoxin expression are increased in the salivary glands of patients with Sjögren's syndrome in response to oxidative stress induced by decreased salivary flow, thus protecting the salivary gland tissues (56). Thioredoxin has also been identified as a biomarker of appetite, with salivary levels modulated by food intake (57).

Oxidations can also occur at the level of methionine residues, generating methionine sulfoxides that can be reduced by methionine sulfoxide reductases (58). In cases of irreversible damage, proteolytic intracellular systems (cytosol proteasome and Lon protease in the mitochondria) eliminate the oxidized polypeptides (59). Many other enzymes involved in oxidation-reduction reactions have been identified from salivary proteomes (26–29). These include protein disulfide isomerases and sulfhydryl oxidase, which catalyze the formation of disulfide bridges, and peroxiredoxins, which catalyze the elimination of peroxides via the oxidation of cysteine residues.

Other salivary proteins may have antioxidant capacity without enzymatic activity mainly by preventing the Fenton reaction due to their metal-binding capacity (60). Salivary histatins have

recently been shown to have antioxidant activity by inhibiting the formation of hydroxyl radicals generated during the Fenton reaction. This activity is likely explained by the chelation of the metal ions Fe^{2+} and Cu^{2+} (61). Histatins are low-molecular weight proteins that represent approximately 30% of the salivary proteins. With likely similar roles, we can also mention albumin (62), ceruloplasmin (63), transferrin (64), and lactoferrin (65).

Oxidative Damage Repair Systems

If the salivary redox state is out of balance leading to the ROS accumulation, biomolecule damages can occur. Depending on the nature of the damaged molecules, different repair systems are involved. In addition to the proteins discussed in the previous paragraph, lipids and DNA can also be affected. These systems, which involve enzymes that are not salivary but whose role is important for oral epithelial cell integrity, are briefly presented in the following lines. ROS can damage unsaturated lipids in the cell membranes by forming highly reactive radical products that are rapidly spreading through the formation of lipid peroxides (R-O-OH). In particular, vitamin E plays a major role in terminating the spread of damage and preserving membranes (66). Membrane-oxidized phospholipids can be specifically recognized and removed by lipolytic enzymes such as phospholipase A2 (67).

DNA is also sensitive to oxidation. Oral epithelial cell genomic alterations can be caused by oxidative stress generated by environmental pollution (68). Many DNA repair systems have been described (13). The diversity of the repair processes is related to the diversity of existing damage and the corresponding severity, as well as the need to preserve the integrity of the genetic information contained in the DNA. The main enzyme systems involved are basal excision repair (BER) for the repair of non-bulky lesions, nucleotide excision repair (NER) for the removal of bulky lesions affecting the three-dimensional structure of the DNA double helix and mismatch repair (MMR).

Methodologies for Characterizing Salivary Redox Status

Salivary redox status is complex and involves many different types of molecules, making difficult its characterization. The most common biomarkers of oxidative stress reactions in the mouth are compounds resulting from lipid peroxidation, protein oxidation, and DNA oxidation and fragmentation products. Consequently, a first approach is to measure these oxidative stress biomarkers. As an example, malondialdehyde is a lipid oxidation product commonly measured as a salivary biomarker that results from the oxidation of polyunsaturated fatty acids (46). Malondialdehyde is titrated by its reaction with thiobarbituric acid, leading to the production of a compound absorbing at 535 nm (69).

A second approach to characterize salivary redox status is to measure the total antioxidant capacity and/or the different antioxidant molecules present in the saliva based on measuring the inhibition of free radical species formation. This method is based on the global measurement of the amount of reducing compounds (without distinction between chemical or enzymatic mechanisms). Total antioxidant capacity is defined as the sum of the antioxidant molecule activities in the saliva. Because these markers are involved in different biochemical pathways in human tissues, their concentrations are not necessarily correlated with each other (70). Thus, the use of a wide range of biomarkers can provide a better understanding of total antioxidant capacity (46).

Approaches to measure the total antioxidant capacity include the ferric reducing-antioxidant power (FRAP) test. This test quantifies the ability of saliva to reduce a colored Fe^{3+} complex by electron transfer to its corresponding Fe^{2+} complex. This method is based on measuring the formation of Fe^{2+}- 2,4,6-tripyridyl-Striazine (TPTZ) or Fe^{2+}-2,3-bis(2-pyridyl)-pyrazine (DPP) complexes, both absorbing at 593 nm, in the presence of saliva (71).

The amount of uric acid is another way to measure the total antioxidant capacity. Indeed, uric acid is both a preventive antioxidant (chelating activity) and a free radical scavenger. The measurement of uric acid is based on hydrogen peroxide production resulting from uric acid in the presence of the enzyme uricase. Hydrogen peroxide is quantified from the formation of the chromophore quinoneimine that absorbs at 500 nm (72). This chromophore is formed during the oxidation of p-hydroxybenzoate and 4-aminoantipyrine in the presence of peroxidase.

Antioxidant capacity measurements in Trolox© equivalent (TEAC) evaluate the combined action of the different free radical scavengers present in saliva. This method is based on the scavenging of the radical cation 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) ($ABTS^{\bullet+}$). The formation of $ABTS^{\bullet-}$ results from the activity of a peroxidase (metmyoglobin or horseradish peroxidase) in the presence of hydrogen peroxide and 2,2'-azobis-(2-amidinopropane) (LABA) and may be followed by measurement of the absorbance at 734 nm. The solution to be analyzed is added after the start of the reaction, and its $ABTS^{\bullet-}$ scavenging activity is compared with that of a reference molecule, Trolox (73). Other methods for measuring the free radical scavenging ability of the saliva have also been described (74, 75).

Glutathione can exist in two forms: reduced (GSH) and oxidized (GSSG). Enzymes that use glutathione (cited in the precedent paragraph) modulate the ratio between these two forms (GSH/GSSG). The titration of these two forms allows the evaluation of their ratio in saliva. This method is based on the combined measurement of total and reduced glutathione. Reduced glutathione is quantified by reaction with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) forming the yellow 5′-thio-2-nitrobenzoic acid (TNB) derivative that absorbs at 412 nm. Measurement of the total amount of glutathione requires reduction of oxidized glutathione to GSH by a glutathione reductase in the presence of NADPH (76). The combination of these two methods therefore enables reduced glutathione and total glutathione quantification and thus one can deduce the amount of oxidized glutathione. Other methods based on electrochemistry can also be used to characterize reduced and total glutathione levels (77).

Importantly, the pre-analytical element of the experiment is crucial and should be taken into account when analyzing the results (78). Indeed, the variations in results between

several studies may originate from pre-analytical differences. For instance, a common practice is to centrifuge the saliva before storing it at −20°C: however, a recent study demonstrated that some salivary enzyme activity may be lost during this step, leading to a reduction in inter-individual differences (9). Each protein eliminated by centrifugation that contains cysteine residues (i.e., with a reducing potential) can contribute to FRAP and TEAC indices. Each method has specific limitations: however, for each method the oxidation of the sample by air oxygen presents a major bias in addition to the loss of salivary protein activity.

RELATIONSHIP BETWEEN SALIVARY ANTIOXIDANT CAPACITY AND PHYSIOLOGIC STATUS

The Physiology of Saliva Secretion

Saliva is secreted by three major glands and numerous minors' glands, such as the von Ebner's glands. The major salivary secreting glands are the parotids, the submandibular and the sublingual. The origins of saliva are actually more complex because it is also composed of gingival fluid and the transudate of the oral and nasal mucosa (79). Saliva also contains bacteria and their metabolites, erythrocytes and cells resulting from the desquamation of the oral mucosa. Salivary gland secretions are neuronally and hormonally controlled (80). In non-pathological conditions, the salivary flow is between 0.75 and 1 L per day. Saliva is an aqueous fluid mainly composed of water that contains numerous organic and inorganic compounds, including salivary proteins that have numerous functions. Salivary pH is between 6.2 and 7.4 (81). As part of the saliva is the result of blood filtration, it can reflect the physiological status of the organism and is often called the "mirror of the body" (82). Indeed, the local vasculature of the salivary glands, the flow of gingival fluid and also intra-oral bleeding allow the passage of compounds from the systemic circulation into the saliva (83). Thus, the salivary concentration of compounds can reflect or can be directly correlated with systemic analytes (82). Moreover, saliva collection is non-invasive and does not required trained staff. Thus, saliva has been thought to be a good candidate to study the global redox status of the organism. However, saliva may not reflect the global physiological status of the organism, as it is a dynamic fluid and food diet and oral microbiota can impact on the salivary redox status.

Parotid saliva is the main source of antioxidants in the saliva. Parotid saliva contains much higher concentrations of various salivary molecules (uric acid) and enzymatic antioxidants (SOD and peroxidases) compared to the saliva secreted by the submandibular/sublingual glands (84). The parotid glands contribute to 20% of the total salivary flow, which is increased up to 60% under stimulated conditions. Thus, one can hypothesize that the higher antioxidant capacity of parotid saliva is aimed at combating deleterious foreign free radicals that may penetrate the body while eating. These observations also suggest that the oral cavity is less protected against ROS in resting conditions, which could increase the damage caused by smoking for instance (84).

Saliva could also have a role in the protection against lipid peroxidation. Indeed, parotid saliva has the ability to reduce peroxide of fatty acids (85). During the gastric phase, the pH of the gastric liquid enhances lipid peroxidation, which is catalyzed by the presence of food compounds such as Fe^{2+} or metmyoglobin in muscle tissues (86). In this condition, the presence of saliva allows partially inhibits lipid peroxidation (87).

This antioxidant role of saliva should also be considered for the formulation of salivary substitutes that could be used for patients presenting salivation troubles. For example, formulations using vegetable mucilage have shown interesting potential with regards to their viscoelastic and antioxidant properties. These substitutes clearly demonstrated their ability to scavenge ROS and chelate metallic ions (88).

Relationship Between Physiological Status and the Antioxidant Capacity of Saliva

The antioxidant capacity of saliva decreases with age (89, 90). Moreover, structural changes are also observed with a decrease in salivary flow (91). These changes lead to increased oxidative stress in the oral cavity. Conversely, aerobic exercise decreases oxidative stress by increasing the concentration and the activity of salivary proteins that present antioxidant activity (92), such as salivary peroxidases (93).

Several studies have reported that obese people have a higher salivary total antioxidant capacity than normal weight individuals (10, 94, 95). Obese individuals reportedly have a higher salivary level of ferric-reducing antioxidant power, indicating a higher capacity of the saliva to chelate and inactivate metal ions (mainly Fe^{2+}) involved in the formation of highly reactive ROS/RNS, including hydroxyl radical, and lipid peroxidation (95). In contrast, peroxidase activity in obese individuals has been described to be lower (94). At the same time, obese individuals show higher oxidative stress that is demonstrated by higher lipid peroxidation (95). Thus, the higher total antioxidant capacity in obese people is hypothesized to counterbalance their high oxidative stress (95).

Relationship Between Oral Microbiota and Salivary Oxidative Stress

Microbiota and the total antioxidant capacity of saliva impact each other. Some microorganisms have the ability to limit the oxidative stress effect by producing antioxidant enzymes (96), while others produce ROS to limit the growth of other species and occupy the ecological niche: however, oxidative stress markers in the saliva are difficult to correlate with specific oral bacteria. No universal answer exists as to which specific bacterial species are associated with ROS production: the marker-species pairs can have negative correlations in some individuals while positive in others. Distinct intra-individual correlation patterns suggest that different bacterial consortia might be at the origin of oxidative stress induction (70). This hypothesis can be explained by the fact that cells belonging to the same bacterial species are distributed in different compartments of the oral cavity with different physiological optimums. While some cells actively metabolize nutrients, others wait for optimal growth conditions

in a dormant stage (70). The microbiome composition can also change during the day (97). Thus, studies on the relationship between oxidative markers and bacteria composition require the collection of multiple samples per individual at different times during the day. The consumption of fermented foods, such as cheese, can lead to a temporary modification of the composition of the microbiota and is associated with a temporary increase in oxidative stress (98). Other studies have demonstrated a positive correlation between the intake of simple carbohydrates and the salivary total antioxidant capacity (99). This observation could be due to increased uric acid in the blood (100, 101). This correlation could also result from an increase in bacteria involved in dental caries formation, leading to an increase in oxidative stress, which could be controlled by increased levels of antioxidant species in the saliva (99).

LINKS BETWEEN SALIVARY REDOX, DIET AND FOOD PERCEPTION

Relationship Between the Total Antioxidant Capacity of Saliva and Diet

Numerous analyses have revealed links between salivary oxidative stress and the pathologies of the oral cavity, including cancers (27, 102), odontogenic cyst (103), lichen planus (104), Sjögren's syndrome (56), and chronic periodontitis (105, 106), leading to the exploration of whether food containing antioxidant compounds or diet supplementation can modulate oral antioxidant capacity (see **Table 2**). **Table 2** presents a list of food and food molecules for which an antioxidant effect has been reported. Most studies have analyzed vitamin supplementation and the effect of food rich in polyphenols.

Indeed, as vitamins are involved in the regulation of the oxidative stress, different studies have investigated the impact of vitamin supplementation with contradictory results. Vitamin C, also known as ascorbic acid, is an antioxidant naturally present in saliva at 2.5 μg/mL. Vitamin C is involved in a variety of hydroxylation reactions. Kamodyova et al. observed a positive effect of vitamin C supplementation (250 mg) on the salivary antioxidant status using two tests, the TAC (TEAC test; average increase of 1%; $p < 0.01$) and on the ability of saliva to chelate and inactivate metal ions (FRAP test; average increase of 107%; $p < 0.01$). At the same time, vitamin C supplementation had a negative impact on carbonyl stress (markers of advanced glycation end products; average decrease of 64%; et $p < 0.001$) (107). Other studies reported that the level of vitamin consumption does not modify antioxidant markers in the elderly (108, 109). The effect of omega-3 fatty acids has also been investigated and revealed no effect on the level of SOD enzymes (110).

Polyphenols, known for their antioxidant properties, are ubiquitous in plant and plant-based foods, and more particularly in tea and berries. Indeed, the antioxidant activity of plants depends on their concentration of polyphenols, and tea and berries contain high polyphenol levels (111). Two mechanisms could be involved in the antioxidant capacity of polyphenols: (i) scavenging of ROS (112) and RNS (113, 114) and ion chelation (115). The chelation of Fe^{2+} ions by polyphenols increases their oxidation to Fe^{3+} ions in the presence of oxygen. This effect depends on the polyphenol structure and is increased when Fe^{2+} ions are bound to a galloyl group (116). The chelation of Fe^{2+} ions with their oxidation to Fe^{3+} ions decreases the quantity of Fe^{2+} that could participate in the Fenton reaction that is at the origin of the production of hydroxyl radicals (116). Thus, red wine tannins inhibit lipid peroxidation of muscle tissues during the gastric phase, whereas peroxidation is only partially inhibited in the presence of saliva alone (87): however, the interaction between polyphenols, especially those having galloyl groups, and salivary proteins, in particular proline rich-proteins (117) or histatins (118), could decrease the antioxidant activity of polyphenols by competing with their binding to Fe^{2+} (109, 119). The effect of polyphenol binding to salivary proteins is not so simple. The interactions of salivary proteins, including mucin, with polyphenols can increase the antioxidant activity of lipophilic polyphenols (120–122) by increasing their solubility. Additionally, these interactions could allow some polyphenols to remain in the oral cavity several hours after consumption (122). This mechanism could be particularly important if the proteins composing the mucosal pellicle are involved, as they are anchored at the surface of the oral epithelial cells (123) and are not swallowed. Monomers of flavan-3-ol also have the ability to bind to lipids present in food and membrane cells and to protect them from oxidation (124).

Many studies have examined the impact of polyphenol rich foods on the antioxidant capacity of saliva. For instance, green tea has been particularly studied because it contains high levels of the oligomers of flavan-3-ol. Green tea reportedly increases the total antioxidant capacity of saliva in several specific populations: chemical laboratory workers (FRAP method; 22% increase; $p = 0.016$) (125), the elderly (TEAC method; 42% increase; $p < 0.001$) (126) and smokers (FRAP method; 43% increase; $p < 0.001$) (127). Green tea intake appears to partially compensate for differences in TAC between smokers and non-smokers (127). In rats, resveratrol intake can protect salivary glands and salivary proteins, including SOD, against the negative effects of irradiation (an important source of oxidative stress) (128). Conversely, another study reported that intake of cranberry juice, which is rich in polyphenols, does not impact systemic or salivary TAC (129).

In addition to polyphenols, oxidant molecules can be present in food, such as caffeine in coffee. Caffeine inhibits salivary aldehyde dehydrogenase, which has an antioxidant function and participates in the detoxification of toxic aldehydes within the oral cavity (130). In response, the secretions of salivary aldehyde dehydrogenase and glutathione transferase are increased (131). This mechanism, which leads to increased secretion of glutathione transferase, has also been observed for Brassicaceae intake including broccoli (131). Red wine is another example of food containing both pro- and antioxidant compounds. The presence of polyphenols in red wine may be able to counteract, at least in part, the pro-oxidant activity of ethanol (132). Ethanol is metabolized by alcohol dehydrogenase (ADH) and cytochrome P450 into acetaldehyde (ethanal) and NADH or acetaldehyde and ROS. Acetaldehyde can inhibit the activity

TABLE 2 | List of food and food molecules that have an antioxidant effect.

Type	Molecules or food	Effects	References
Food	Apple	Inhibit nitrosation/nitration but also promote NO bioavailability at the gastric level	(163)
Food	Broccoli and coffee	Increase in the secretion of glutathione transferase	(131)
Food	Bryndza cheese	Decrease in salivary TAC	(98)
Food	Coffe and Chlorogenic acid	Decrease in RNS	(113)
Food	Cranberry	No effect on salivary total antioxidant status	(129)
Food	Green Tea	Increase in the salivary TAC in chemical laboratory workers	(125)
Food	Green Tea	Increase in the salivary TAC in elderlies	(126)
Food	Green Tea	Increase in the salivary TAC in smokers	(127)
Food	Green Tea	No effect on free radical scavenger activity after exercise training	(164)
Food	Polyphenols in red wine	Offset in the oxidative impact of ethanol	(132)
Food	Sea buckthorn oil extract	No effect on oxidative markers and salivary flow	(165)
Food	Tannins	Increase in the secretion of histatines	(136)
Food	Wine	Presence of polyphenols in wine preclude the oxidative effect of ethanol	(132)
Molecules	Astaxanthine	Decrease in the level of salivary oxidative stress via ROS scavenging	(141)
Molecules	Caffeine	Inhibit human salivary aldehyde dehydrogenase	(130)
Molecules	Flavan-3-ol	Galloyl-containing polyphenols promote iron oxidation at a significantly faster rate than analogous catechol-containing compounds, and iron oxidation rate also correlates with polyphenol inhibition of DNA damage for polyphenol compounds	(116)
Molecules	Isoflavone supplementation in addition to combined exercise	Decrease in nitrite and thiobarbituric acid reactive substances and no effect on total antioxidant capacity or total protein	(166)
Molecules	Nitrate	Increase in nitrite and uric acid concentration in saliva	(142)
Molecules	Omega-3-fatty acids	No effect on the SOD level in saliva	(110)
Molecules	Quercetin	Enhanced the formation nitric oxide	(113)
Molecules	Quercetin	Reduction of nitrous acid to nitric oxide	(114)
Molecules	Resveratrol	Protect salivary glands against the negative effects of irradiation	(128)
Molecules	ß-carotene	No effect on salivary TAC and C- reactive proteins	(108)
Molecules	ß-carotene	No effect on Non-Urate Total Antioxidant Capacity in elderlies	(109)
Molecules	Vitamin C	Increase in the antioxidant status; Decrease in carbonyl stress	(107)
Molecules	Vitamin C	No effect on TAC	(167)
Molecules	Vitamin C	No effect on salivary TAC and C- reactive proteins	(108)
Molecules	Vitamin C	No effect on Non-Urate Total Antioxidant Capacity in elderlies	(109)
Molecules	Vitamin C	No effect on the SOD activity in saliva	(168)
Molecules	Vitamin E	No effect on salivary TAC and C- reactive proteins	(108)
Molecules	Vitamin E	No effect on Non-Urate Total Antioxidant Capacity in elderlies	(109)
Molecules	Vitamin E	No effect of SOD activity in saliva	(169)

of salivary peroxidases (lactoperoxidase and myeloperoxidase) (133). As a result, an increase in the activity of salivary peroxidases correlated to a decreased salivary flow in alcoholic patients (134). Whether this increase in salivary peroxidase activity aims at compensate its inhibition by acetaldehyde or if it originates from an influx of leukocytes into damaged oral mucosa remains unknown (134).

Moreover, the effect of polyphenols could be indirect. In mammals, a tannin rich diet induces increased secretion of tannin-binding salivary proteins in the saliva of herbivores (135, 136). Tannin-binding salivary proteins are mainly composed of two salivary protein families: proline-rich proteins and histatins (137). These proteins have demonstrated a high affinity for tannins (118, 138) due to their structure (117, 139) and play a role in the protection of the oral mucosa (140). Histatins have recently been shown to have antioxidant activity, as they are able to decrease the production of hydroxyl radical likely through the binding and scavenging of Fe^{2+} ions or through Fe^{2+} oxidation to Fe^{3+} (61). Thus, an increased histatin concentration in the saliva could increase the antioxidant capacity of the oral cavity.

Carotenoids are another class of molecules present in plants and animals that have antioxidant properties that could impact salivary antioxidant capacity. For instance, astaxanthin, a carotenoid with strong antioxidant properties, is found in fishes such as sea bream and salmon as well as crustaceans such as crab and shrimp. Diet supplementation with astaxanthin induced a decrease in oxidative stress at the salivary level (measurement of the lipid peroxidation marker hexanoyl-lysine; 10% decrease;

$p = 0.03$). This molecule appears to scavenge ROS (141). A rich nitrate (NO_3^-) diet can also affect the antioxidant capacity of saliva. Nitrate is ingested as a food component. The nitrate ingested is absorbed from the intestine into the bloodstream and is then secreted into the saliva. In the saliva, nitrate is reduced to nitrite (NO_2^-) by certain bacteria, and the nitrite formed is reduced to nitric oxide (NO). Then, nitric oxide can react with molecular oxygen to produce NO_2 and N_2O_3.

$$2NO + O_2 \rightarrow 2NO_2$$
$$NO_2 + NO \rightarrow N_2O_3$$

NO can also react withO_2^- that is produced by bacteria forming $ONOO^-$.

$$NO + O_2^- \rightarrow ONOO^-$$

NO_2 and $ONOO^-$ are oxidizing and nitrating agents and thus reactive species. In acidic conditions, nitrite can be protonated and form nitrous acid, which decomposes to various nitrogen oxides. The RNS generated contribute to oxidative stress in the oral cavity (113, 114): however, polyphenols, such as quercetin, are able to reduce nitrous acid to nitric oxide (113). A study on the impact of nitrate supplementation after physical exercise for 5 days reported an increase of nitrite and uric acid in the saliva. Moreover, lipid peroxidation and SOD activity were decreased after 30 min in supplemented subjects. The decreased lipid peroxidation can be explained by the increased NO resulting from the nitrite, which acts as an inhibitor of lipid peroxidation by scavenging peroxyl radicals (142).

Last, eating, especially mastication, plays an indirect role in salivary antioxidant capacity. The parotid glands of experimental animals fed with a liquid diet reportedly show atrophy (143). Parotid gland atrophy is due to decreased parasympathetic nerve stimulation, which is involved in the proliferation of the parotid glands (144). Thus, a lack of sufficient masticatory force might lead to a reduced masticatory-parotid reflex and consequently lead to atrophy of the salivary glands. Parotid gland atrophy leads to decreased parotid salivary flow and thus to salivary antioxidant capacity. Indeed, enteral nutrition feeding alters salivary antioxidant capacity by decreasing the concentration of uric acid and the total protein content in the saliva (145). Another study demonstrated that children with eating difficulties resulting from enteral or parenteral nutrition in neonatal periods have a lower antioxidant status (146): however, supplementation of a liquid diet with L-carnitine in rat prevented atrophy of the parotid glands. L-carnitine has been hypothesized to protect the mitochondria and endoplasmic reticulum from oxidative stress that results from decreased cellular energy production (147). Being a potential scavenger of ROS, L-carnitine is an antioxidant that prevents the impairment of fatty acid oxidation in the mitochondria.

Relationship Between Food Flavor Perception and Salivary Antioxidant Capacity

During the last decade, several studies have considered the role of salivary antioxidant capacity in flavor perception. Indeed, flavor molecules are subject to oral and nasal metabolization as a function of their structure. These reactions affect both the quality and the quantity of flavor compounds available to activate the chemosensory receptors. Different results have suggested that these reactions may be modulated by salivary antioxidant capacity (8–10). A recent article showed a highly significant positive correlation between salivary TAC and taste disorder in 120 patients compared to normal subjects. Increased TAC was associated with increased catalase and SOD activities (148).

The interest to gain a deeper understanding of these mechanisms has grown with the recent demonstration that nasal and oral metabolic activity of aroma compounds impact aroma perception (12, 149). Thus, several studies have investigated the impact of salivary antioxidant capacity on the perception and release of aroma compounds in addition to the role in fatty acid perception.

Several studies on both normal subjects (9) and specific populations including obese (10), elderly (11) or the elderly suffering from hyposalivation (8) have observed a negative correlation between salivary TAC and aroma release in the presence of the subjects' saliva. This effect depended on the structure of the aroma compounds and was related to the chemical reactivity of the molecules. Whereas, some ketones and aldehydes were strongly affected, the studied alcohols were not metabolized (9). Indeed, Muñoz et al. suggested that the decreased release of specific aroma compounds results from their metabolization by salivary xenobiotic-metabolizing enzymes. Xenobiotic-metabolizing enzymes are involved in the clearance and deactivation of xenobiotics, including aroma compounds. This effect is achieved through two enzymatic steps involving different classes of xenobiotic-metabolizing enzymes. Phase I enzymes catalyze the biotransformation of compounds with reactive chemical functions, including carbonyl groups, forming metabolites carrying less reactive functional groups (OH, NH_2, COOH). Phase II enzymes, including glutathione transferases (GST), can promote the biotransformation of compounds naturally functionalized or phase I metabolites by catalyzing their conjugation with hydrophilic moieties (e.g., glutathione). Phase II leads to hydrophilic conjugates of the initial molecules, including glutathione conjugates of some flavor compounds such as cinnamaldehyde (150). Some of these enzymes are NAD(P)H dependent (9). The TAC provides information on the redox status and indirectly on the equilibrium of the balance of $[NAD(P)^+]/[NAD(P)H]$ and thus on the level of activity of these enzymes. Indeed, addition of NADH to the saliva significantly increased the enzymatic degradation of octanal into octanol (9). This metabolic activity has also been reported in the presence of oral epithelial cells (151). Coffee or broccoli intake has been reported to increase the secretion of xenobiotic-metabolizing enzymes, including GST, resulting in a higher metabolic activity at the salivary level (131). This mechanism is believed to impact perception by modulating the quality and quantity of flavor compounds (6).

Beside this metabolic activity, lipid oxidation in the oral cavity leads to the formation of volatile aldehydes and ketones that can have a metallic aroma note and as a result participate in the perception of a metallic taste. In fact, metallic taste is

more a flavor as it probably results from the cerebral integration of different senses leading to a multimodal perception. Lipid oxidation, especially of polyunsaturated fatty acids, is highly increased in the presence of Fe^{2+} ions and to a lesser extent by Fe^{3+} (152). This oxidation is precluded in the presence of molecules that are able to chelate Fe^{2+} ions, and only partially reduced in the presence of vitamin C, an antioxidant that is able to scavenge free radicals (152). Indeed, vitamin C can also reduce Fe^{3+} to Fe^{2+}, which will participate to the formation of ROS via auto-oxidation or the Fenton reaction (153, 154): however, no correlation between the salivary TAC, metallic taste perception and the production of aldehydes and ketones resulting from lipid oxidation has been observed (152).

Concerning fat perception, Poette et al. have reported a positive correlation between the detection threshold of non-esterified fatty acids without nose-clip and the antioxidant status of saliva, whereas there is no correlation with nose clip (155). These results suggest that the metabolization or the oxidation of non-esterified fatty acids leads to the release of aroma compounds involved in fat perception. Thus, as for metallic taste, fat perception appears to be a multimodal perception involving both the perception of aroma compound release in the oral cavity, trigeminal perception (mechanoreceptors involved in the perception of texture) and potentially taste chemoreceptors dedicated to the detection of free fatty acids such as the CD36 receptors located in the taste buds (156).

CONCLUSION

Salivary antioxidant capacity involves numerous molecular species that can counterbalance their respective activity. Moreover, feeding and associated pathologies (obesity, alcoholism, etc.) can increase salivary oxidative stress, while the consumption of antioxidants can increase salivary antioxidant status: however, the direct association of foods with salivary antioxidant capacity remains fragile and contradictory results on the effect of antioxidant intake highlight the necessity for future studies in this area, which should consider more than one antioxidant marker to account for the different biochemical mechanisms. Such investigations should provide a deeper understanding of the respective contribution of the different mechanisms involved. Another promising area of research concerns the relationship between salivary antioxidant capacity and the perception of flavor. Indeed, flavor corresponds more to the perception of the sum of flavor molecules and metabolites formed in mouth than food flavor molecules alone. Highly toxic flavor compounds are hypothesized to be quickly eliminated by xenobiotic-metabolizing enzymes at the salivary level before they reach the chemosensory receptors. Thus, the metabolites formed by the detoxification pathway can themselves provide information on the potential toxicity of food. Several markers of salivary antioxidant status should be considered to preserve the interindividual variability of saliva.

AUTHOR CONTRIBUTIONS

MS, FN, and FC drafted the manuscript with input from all authors. GF, MS, FN, and FC revised the manuscript. All authors have approved the submitted version.

FUNDING

This work was supported by the Agence Nationale de la Recherche (ANR), grant number ANR-20-CE21-0002, ANR-16-CE21-0004-01 and ANR-14-CE20-0001-01.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 01 February 2021
doi: 10.3389/fnut.2020.616484

Factors Associated With Food Texture Acceptance in 4- to 36-Month-Old French Children: Findings From a Survey Study

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OPEN ACCESS

Edited by:
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Reviewed by:
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Francis Kweku Amagloh, University for Development Studies, Ghana

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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *12 October 2020*
Accepted: *15 December 2020*
Published: *01 February 2021*

Citation:
Tournier C, Demonteil L, Ksiazek E, Marduel A, Weenen H and Nicklaus S (2021) Factors Associated With Food Texture Acceptance in 4- to 36-Month-Old French Children: Findings From a Survey Study. Front. Nutr. 7:616484. doi: 10.3389/fnut.2020.616484

Food texture plays an important role in food acceptance by young children, especially during the complementary feeding period. The factors driving infant acceptance of a variety of food textures are not well-known. This study summarizes maternal reports of children's ability to eat foods of different textures (here: acceptance) and associated factors. Mothers of 4- to 36-month-old children (n = 2,999) answered an online survey listing 188 food-texture combinations representing three texture levels: purees (T1), soft small pieces (T2), hard/large pieces, and double textures (T3). For each offered combination, they reported whether it was spat out or eaten with or without difficulty by the child. A global food texture acceptance score (TextAcc) was calculated for each child as an indicator of their ability to eat the offered textured foods. The results were computed by age class from 4–5 to 30–36 months. The ability to eat foods without difficulty increased with age and was ranked as follows: T1 > T2 > T3 at all ages. TextAcc was positively associated with exposure to T2 (in the age classes between 6 and 18 months old) and T3 (6–29 months) and negatively associated with exposure to T1 (9–36 months). Children's developmental characteristics, as well as maternal feeding practices and feelings with regard to the introduction of solids, were associated with texture acceptance either directly or indirectly by modulating exposure. Children's ability to eat with their fingers, gagging frequency, and to a lesser extent, dentition as well as maternal feelings with regard to the introduction of solids were the major factors associated with acceptance. This survey provides a detailed description of the development of food texture acceptance over the complementary feeding period, confirms the importance of exposure to a variety of textures and identifies a number of additional person-related associated factors.

Keywords: eating ability, chewing skills, infant, parental report-based measures, food texture, complementary feeding, feeding practices

INTRODUCTION

Early childhood is a period of rapid growth and plays a critical role in the development of health outcomes (1). Dietary experiences during this period are critical from both nutritional and developmental points of view because they shape eating habits during later childhood and even adulthood (2, 3). It is therefore important to fully understand the dietary experiences that promote healthy eating habits, especially during the complementary feeding (CF) period when a large variety of foods other than milk are introduced to the infant's diet. In this context, the development of food texture acceptance during the course of CF merits scrutiny. Indeed, the texture of food plays a crucial role in food rejection and contributes to feeding difficulties in children (4–7). Food texture acceptance develops with age throughout the course of the CF period and is related to the development of children's oral-motor skills. The development of these skills ensures an effective transition of the child's diet toward the foods from the family table. However, a detailed description of the development of children's acceptance across the CF period and for a variety of textures has not yet been provided.

Acceptance of food texture requires children to have the ability to chew and swallow food. Children's chewing has been investigated in experimental studies using different methods, such as the evaluation of video recordings of infants eating foods (4, 8, 9), the monitoring of chewing muscle activity and jaw movements (10–12) or the determination of particle sizes of boluses collected under standardized conditions (13). Children's acceptance has been determined from observation of ingestive behavior; a food is considered accepted when it is eaten by the child. The acceptance level of various textured foods was defined from the mean percentage of children of a given age swallowing a small quantity/piece of these foods (14) or from food intake (in grams or number of spoons) (7, 15, 16).

Various individual factors were suggested to influence food texture acceptance in infants and toddlers. The primary factor is the degree to which children have been exposed to a diet of varied food textures. Early exposure to a large variety of textures after the initiation of CF stimulates the development of oral-motor skills and facilitates the acceptance of more complex textures (15, 17–19). Other maternal feeding practices, such as breastfeeding or eating the same foods as the family, are also thought to be favorable for texture acceptance (20). Texture acceptance is also modulated by individual eating temperament (15, 16) and tactile sensitivity (21–23). Dentition is thought to play a role in the development of chewing ability in 9-to-36-month-old children (11), and the number of teeth was positively associated with the *ad-libitum* intake of chopped carrots among 12-month-old children (15). Finally, individual differences in developmental factors possibly associated with readiness to eat food pieces (e.g., ability to sit alone, ability to eat alone with fingers or with a fork) have not been studied specifically but probably also play a role in children's acceptance of solid foods.

Abbreviations: TextAcc, Texture acceptance score; CF, complementary feeding.

Whereas, experimental studies are the most objective and controlled way to evaluate the development of food texture acceptance, they are limited to a small subset of foods and children (4, 14–16). In addition, the laboratory environment and/or the process of being observed may alter the child's eating behavior compared to the daily situation at home (24). The alternative is to study food texture acceptance in a survey, which does not have these disadvantages and allows a larger number of subjects. This makes it possible to study a number of factors together and compare them. A study based on parental reports of children's ability to eat specific foods would make the evaluation of texture acceptance possible (1) for the textured foods introduced to children's diet, (2) to compare eating ability at specific time points during the entire CF period, and (3) to assess factors associated with interindividual variability at a given age. Parental self-reports have been used in many studies to assess different facets of children's eating behavior [baby and children eating behavior (25, 26) or eating difficulties (6, 27)] with success. However, few attempts have been made to evaluate the ability to eat food texture using this approach. A previous study (28) conducted in-home interviews to assess the oral-motor development of children between 2 and 24 months. Mothers reported the child's age when specific behaviors (eating food with tiny lumps, chewing and swallowing firmer foods without choking, etc.) first occurred. Another previous study (29) used an open-ended survey for parental reports of food textures that are "easy" or "difficult" to eat for their child with Down syndrome. Finally, Sakashita et al. (20, 30) proposed a detailed questionnaire containing food items offered during CF in Japan for which parents evaluated their child's eating ability. To date, no questionnaire has been reported to assess children's ability to eat textured foods offered during CF in France, where children have been reported to be exposed to textured foods only to a limited extent before 12 months (31) and where texture introduction is a matter of concern for some parents (32).

The objective of this work is to evaluate the development of infants' and toddlers' ability to eat a variety of food textures using a cross-sectional study. In a previous publication (31), we reported data showing the course of introduction of foods in children aged between 4 and 36 months old. In the present work, we studied parental evaluation of their child's ability to eat the foods they introduced, representing a large range of textures. Specifically, the first objective is to report the evolution of food texture acceptance with age. The second objective is to study the individual factors associated with acceptance among children of a given age. It was hypothesized that older children display a better ability to eat foods with different textures than younger ones, that food acceptance (as reported by parents) would be positively associated with dietary exposure to a variety of textures and that acceptance would be positively related to children's number of deciduous teeth and feeding skills.

MATERIALS AND METHODS

Data were collected using a survey conducted with parents of French children aged 4–36 months, aiming to describe

both parental feeding practices with regard to food texture introduction and texture acceptance by children. The cross-sectional survey was launched online through a large database of members of the web information programme of the Blédina brand [declared to the national data protection authority, the Commission Nationale Informatique et Liberté (CNIL), no. 1824320v0] from September to December 2015. The survey was approved by the local ethics committee (Comité de Protection des Personnes Est III, no. 2015-A00323-46).

Description of the Survey

The first part of the survey collected information on maternal characteristics (age, country of birth, education level, source of information for advice on CF practices) and children's characteristics (sex, birth order, measured birth and current weight and length, number of teeth). Birth and current weight-for-length z-scores were determined using the World Health Organization child growth standards (33). Parents evaluated their children's motor skills (sitting up alone, pacifier use, thumb sucking, drooling) and feeding skills/behaviors (eating with fingers, self-feeding with a fork, gagging when food or object enter the mouth) using a 4-category scale: "never," "rarely," "sometimes," and "often." Reported maternal feeding practices included breastfeeding (yes/no), age at CF introduction, letting the child participate in family meals (yes/no), attendance to day care meals (yes/no), practice of baby-led weaning (BLW, yes/no) and the type of food preparation for their child ("exclusive use of ready-prepared baby food," "exclusive use of homemade foods" or "use of both ready prepared and homemade foods"). Finally, maternal feelings ("eager," "unconcerned," "reluctant") regarding the introduction of solid foods were reported.

The second part of the survey aimed to evaluate acceptance of the solid foods that children had already tried. This part was inspired by the survey developed by Sakashita and collaborator for Japan (20) and adapted to CF practices in France. Parents were shown a list of 61 foods commonly used in France, with each food item presented in different texture formats (puree, pieces, raw, cooked, etc.). A maximum of 188 combinations were shown (**Supplementary Material 1**). For example, for "carrot," the following food-texture combinations were shown: smooth carrot puree, rough carrot puree, cooked carrot in small pieces, cooked carrot in large pieces, raw grated carrot, raw carrot in small pieces, and raw carrot in large pieces. To help parents in their assessments, they were provided with pictures illustrating the size of the pieces with a scale (**Supplementary Material 2**). For each food, mothers were asked to record whether they had already offered it to their child (yes/no). If they introduced the food, they self-reported for each food-texture combination introduced their child's ability to eat this combination by selecting one of the following answers: "*offered but spat out immediately,*" "*chewed but spat out,*" "*sucked and swallowed,*" "*eaten with some difficulties,*" "*eaten without difficulties*." Preliminary analysis showed that some answer categories were rarely selected. Therefore, in the reporting and analysis, some categories were grouped together: "*offered but spat out immediately*" and "*chewed but spat out*" were grouped into "*spat out*" and coded as 0; "*sucked and swallowed*" and "*eaten with some difficulties*" were grouped into "*eaten with difficulties*" and coded as 1; "*eaten without difficulties*" was coded as 2.

Definition of Food Texture Levels and Coding of Acceptance Answers

The 188 food-texture combinations were categorized into three texture levels according to the feeding skills necessary to process the food (see full list and level classification in **Supplementary Material 1**). Smooth and rough purees, which can be processed by sucking motions or limited tongue-palate compressions, were categorized as "simple texture," also called the T1 level. Soft solid textures (small cooked pieces, soft foods) that require more intensive tongue-palate or gum-gum compressions were categorized as "intermediate texture" (T2 level). Last, large cooked and/or hard pieces that require the tongue, the presence of teeth and masticatory movements to be swallowed and double textures (pieces in a thin liquid phase), which require swallowing the liquid phase while maintaining the pieces in the oral cavity for further breakdown, were categorized as "hard/large pieces and double textures" (T3 level). By doing so, among the 188 food-texture combinations in the survey, 39 were classified at the T1 level, 40 at the T2 level and 109 at the T3 level (**Supplementary Material 1**).

Determination of a Food Texture Acceptance Score (TextAcc)

For each child, we determined a food texture acceptance score (TextAcc, **Equation 1**), which is a global indicator of a child's ability to eat food textures and was aimed at comparing children of the same age and identifying factors of the observed differences. We designed the score in such a way that it increased with the level of acceptance (spat out < eaten with difficulties < eaten without difficulty) of given food and with the texture level of this food (T1 < T2 < T3). The score takes into consideration the total number of foods introduced in the child's diet [which is known to vary considerably among children of a given age class (31)]. This score was built as follows: first, the number of food-texture combinations offered to the child was determined for each texture level (NT1, NT2, NT3). Then, an acceptance score was calculated for each texture level from the sum of the acceptance levels (coded 0 {"spat out"}, 1 {"eaten with difficulties"} or 2 {"eaten without difficulties"}) of offered food-texture combinations. These scores were assigned a different weight, depending on the texture level: 1 for the T1 level, 2 for the T2 level, and 3 for the T3 level. TextAcc was finally obtained from the sum of the weighted acceptance scores collected for the T1, T2, and T3 levels divided by the total number of food-texture combinations offered to the child (**Equation 1**).

$$TextAcc = \frac{\sum_{i=1}^{NT1}(acceptance\ level_i \times 1) + \sum_{j=1}^{NT2}(acceptance\ level_j \times 2) + \sum_{k=1}^{NT3}(acceptance\ level_k \times 3)}{NT1 + NT2 + NT3} \quad (1)$$

where T1 is the texture of smooth and rough purees, T2 is soft solid textures, and T3 is the texture of large cooked and/or hard pieces and double textures; i, j, and k: one food-texture combination within the texture levels T1, T2, and T3; NT1, NT2, NT3: the number of food-texture combinations of texture level T1, T2, and T3 offered to the child; acceptance level: acceptance level of a given food-texture combination (0: "spat out," 1: "eaten with difficulties," 2: "eaten without difficulties").

Statistical Analysis

Data were split into 14 age classes in agreement with (31). The split was organized by month during the first 12 months (except for infants of 4 and 5 months, which were grouped together), as the infant's oral skills develop quickly during this period. Above the age of 12 months, responses were split into larger age classes: 13–15, 16–18, 19–21, 22–24, 25–29, and 30–36 months.

Statistical analyses were run using SAS 9.4 (SAS Institute, Inc., Cary, North Carolina). For each age class, we determined the ratio (%) of food-texture acceptance "*spat out*," "*eaten with difficulties*," "*eaten without difficulties*" over the total number of combinations offered within each texture level (T1, T2, T3). The evolution of these ratios with age was assessed using one-way analyses of variance (ANOVAs) and Student Newman-Keuls *post-hoc* analyses to compare mean values. The impact of texture on the ratio was studied for each age class using one-way ANOVAs and Student Newman-Keuls *post-hoc* analyses.

The effect of age class on TextAcc was assessed using ANOVA and the Student-Newman-Keuls test *post-hoc* analysis. The study of factors associated with this score was performed for each age class independently. Associations between TextAcc and 20 variables representing children's characteristics ("sex," "number of teeth," "birth order," "current weight-for-length z-score"), motor and feeding skills ("use of pacifier," "thumb sucking," "drooling," "gagging," "sitting alone," "eating with fingers," "self-feeding with a fork") and maternal feeding practices ("breastfeeding," "age of CF," "T1 exposure score (number of T1 combinations introduced)," "T2 exposure score," "T3 exposure score," "attendance at day care meal," "type of food preparation," "meal taken with the family") and "maternal feeling with regard to the introduction of solids" were studied using separate bivariate linear models. The results from bivariate analysis are presented in **Supplementary Material 3**. Variables significantly associated with TextAcc for at least four age classes were entered in a multivariate linear model, which included the number of T1, T2, and T3 foods introduced, number of teeth, eating with fingers, gagging, age of CF, and maternal feelings concerning the introduction of solid foods corrected for weight-for-length z-score.

RESULTS

Study Population

A total of 3,771 respondents participated in the survey. Data from respondents other than mothers (fathers or grandmothers, $n = 71$), twins ($n = 37$), children born at a gestational age under 37 weeks of amenorrhea ($n = 137$), with severe gastroesophageal reflux ($n = 247$) or tube-fed at birth ($n = 139$), aged below 4 months or above 36 months ($n = 131$) and missing data with regard to food texture introduction ($n = 10$) were excluded, yielding a final sample of 2,999 children. Most of the mothers were born in France (94.6%), their age was 31.1 (*SD* 4.7) years on average, and 65.0% had attained an educational level of 2–3 years of university or more. The characteristics of the children are described in **Table 1**. Children were mainly first-born (77.1%) and balanced in gender (48.1% female).

Children's motor skills evolved as a function of age (**Table 1**). Most of the children (>80%) were reported to be able to sit alone at 8 months, to eat with their fingers at 16–18 months and with a fork at 22–24 months (**Table 1**). The proportion of children having "sometimes/often" gag reflex was ∼30% in 6-to-8-month-old children and decreased to <15% in children aged 19–21 months and older. The frequency of thumb sucking decreased with age (62.7% of 4–6-month-old to 10.5% of 30–36-month-old children), whereas frequent pacifier use was relatively constant across ages (48.4% on average). The number of teeth increased with age (from 0.5 ± 1.9 at 4–5 months to 18.2 ± 2.4 at 30–36 months). For maternal feeding practices, children were on average introduced to CF at 4.9 months, and 62.9% of them were/had been breastfed. At 12 months, the frequency of children taking part in family meals was 33.6%; it then increased to 88.0% in 30–36-month-old children. Mothers very rarely used the baby-led weaning (BLW) method (1.8%). Mothers were mainly feeding their child by using both commercial baby and homemade foods (53%). Exclusive use of ready-prepared baby foods decreased from 22.5% in 4- to 5-month-old children to <10.0% after 15 months. Most mothers were either unconcerned (40.9%) or eager (26.4%) with regards to the introduction of food pieces, whereas 32.7% were reluctant to introduce them.

Pattern of Ability to Eat Different Textures as Function of Age

The mean number of food-texture combinations "offered" and their level of acceptance are presented in **Figure 1**, and the ratios of the number of food texture combinations accepted vs. offered in **Figure 2**. Acceptance (i.e., ability to eat without difficulty) increased with age and was very much related to the offering pattern (**Figure 1**). Acceptance for soft and rough purees (T1 level) significantly increased with age [$F_{(13,2982)} = 36.9$, $p < 0.001$]: it increased from 4/5 months to 7 months (from 70 to 82% of offered T1 combinations) and was relatively stable afterwards (between 87 and 93% in the period from 8 to 30–36 months old) (**Figure 2**). The proportion of small and soft pieces (T2 level) eaten without difficulty is 5 to 10% lower than that of T1 level items. The age effect for the acceptance of small and soft pieces was smaller but still significant [$F_{(11,2437)} = 2.2$, $p = 0.01$; with

TABLE 1 | Characteristics of the participants.

	Age class in months														
	All[a]	**4-5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13-15**	**16-18**	**19-21**	**22-24**	**25-29**	**30-36**
	***N* = 2,999**	***N* = 142**	***N* = 283**	***N* = 235**	***N* = 243**	***N* = 187**	***N* = 195**	***N* = 168**	***N* = 137**	***N* = 370**	***N* = 279**	***N* = 254**	***N* = 178**	***N* = 203**	***N* = 125**
CHILDREN'S CHARACTERISTICS															
Current weight-for-length z-score [mean (sd)]	0.16 (1.2)	−0.01 (1.3)	0.04 (1.3)	0.07 (1.7)	0.16 (1.4)	0.15 (1.3)	0.28 (1.1)	0.28 (1.1)	0.10 (1.4)	0.32 (1.1)	0.29 (1.3)	0.31 (1.3)	0.20 (1.1)	−0.05 (1.0)	−0.15 (1.3)
Number of teeth [mean (sd)]	6.5 (6.3)	0.5 (1.9)	0.4 (1.3)	0.7 (1.5)	1.4 (2.0)	2.4 (2.2)	3.3 (2.5)	3.9 (2.3)	5.4 (2.5)	7.0 (2.9)	10.3 (3.6)	13.4 (3.6)	15.2 (3.1)	16.4 (2.7)	18.2 (2.4)
Girls [*N* (%) or %]*	1,442 (48.1)	51.4	47.0	48.1	48.5	51.3	46.7	42.9	43.8	49.5	50.2	50.0	48.3	49.3	40.0
Birth order [*N* (%) or %]															
1st born	2,290 (77.1)	74.3	84.3	75.1	76.2	72.7	78.2	77.7	74.6	77.1	76.0	73.0	79.1	79.2	81.2
CHILDREN FEEDING SKILLS															
Sitting alone [*N* (%) or %]															
Sometimes/often	2,599 (86.8)	29.8	42.9	66.9	83.9	93.6	99.5	97.6	100.0	99.7	100.0	100.0	100.0	100.0	100.0
Never/rarely	394 (13.2)	70.2	57.1	33.1	16.1	6.4	0.5	2.4	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Drooling [*N* (%) or %]															
Sometimes/often	1,997 (66.6)	96.5	97.2	91.1	92.6	84.0	80.0	81.6	78.8	58.3	53.6	37.4	38.2	23.7	10.5
Rarely/never	999 (33.31)	3.5	2.8	8.9	7.4	16.0	20.0	18.5	21.2	41.7	46.4	62.6	61.8	76.4	89.5
Pacifier using [*N* (%) or %]															
Often	1,451 (48.4)	51.4	47.4	52.8	49.8	55.6	49.7	45.8	48.2	47.7	46.6	48.0	53.4	36.5	46.4
Sometimes/rarely	679 (22.6)	26.1	28.3	22.6	24.3	21.9	24.1	26.2	18.3	25.5	20.4	16.5	19.1	20.7	19.2
Never	868 (28.9)	22.5	24.4	24.7	25.9	22.5	26.2	28.0	33.6	26.8	33.0	35.4	27.5	42.9	34.4
Thumb sucking [*N* (%) or %]															
Sometimes/often	908 (30.3)	62.7	58.0	52.8	50.6	34.8	26.7	26.8	20.4	17.3	16.9	15.8	8.5	19.3	10.5
Rarely/never	2,088 (69.6)	37.3	42.1	47.2	49.4	65.2	73.3	73.2	79.6	82.7	83.2	84.3	91.5	80.7	89.5
Eating with fingers [*N* (%) or %]															
Sometimes/often	1,605 (54.0)	7.2	7.2	10.5	16.6	29.7	43.3	46.7	69.4	74.5	87.8	90.6	88.8	91.6	86.4
Rarely/never	1,368 (46.0)	92.8	92.8	89.5	83.4	70.3	56.7	53.3	30.6	25.5	12.2	9.4	11.2	8.4	13.6
Self-feeding with a fork [*N* (%) or %]															
Sometimes/often	827 (28.0)	1.4	0.0	0.0	0.4	0.6	1.1	3.6	1.5	12.9	45.4	70.1	83.7	96.1	97.6
Rarely/never	2,110 (72.0)	98.6	100.0	100.0	99.6	99.4	98.9	96.4	98.5	87.1	54.6	29.9	16.3	3.9	2.4
Gagging [*N* (%) or %]															
Sometimes/Often	645 (21.8)	27.5	30.9	29.7	32.1	27.4	19.5	24.7	24.6	18.9	17.1	13.4	14.9	9.9	14.0
Rarely	1,050 (35.6)	31.9	32.6	42.7	38.0	37.1	39.5	36.8	37.3	42.7	31.3	35.2	35.1	24.4	24.0
Never	1,258 (42.6)	40.6	36.5	27.6	29.9	35.5	41.0	38.5	38.1	38.4	51.6	51.4	50.0	65.7	62.0
FEEDING PRACTICES															
Any breastfeeding [*N* (%) or %]	1,886 (62.9)	55.6	62.9	63.8	67.1	63.6	63.1	62.5	63.5	56.5	63.4	65.0	67.4	64.0	64.8
Age of CF [mean (sd)]	4.9 (1.1)	4.1 (0.4)	4.4 (0.6)	4.7 (0.7)	4.8 (0.8)	4.9 (0.9)	4.9 (0.8)	4.8 (0.8)	4.9 (1.2)	5.0 (1.0)	5.1 (1.2)	5.0 (1.1)	5.0 (1.1)	5.2 (1.7)	5.1 (1.7)
Meal taken with the family [*N* (%) or %]*	1,227 (40.9)	19.7	14.1	22.6	26.8	26.2	21.0	27.4	33.6	41.1	54.1	59.8	71.4	82.3	88.0
Attendance to day care meal [*N* (%) or %]*	951 (32.0)	18.6	26.4	26.4	28.8	27.8	24.7	30.5	27.7	33.0	41.9	41.1	36.0	37.8	41.0

(Continued)

TABLE 1 | Continued

	Age class in months														
	All[a]	4-5	6	7	8	9	10	11	12	13-15	16-18	19-21	22-24	25-29	30-36
	N = 2,999	*N* = 142	*N* = 283	*N* = 235	*N* = 243	*N* = 187	*N* = 195	*N* = 168	*N* = 137	*N* = 370	*N* = 279	*N* = 254	*N* = 178	*N* = 203	*N* = 125
BLW knowledge [*N* (%) or %]*	289 (9.7)	4.2	8.0	14.3	10.8	11.9	8.5	9.4	8.6	9.2	10.2	8.0	9.4	10.6	11.7
BLW application [*N* (%) or %]*	55 (1.8)	0.0	0.0	2.5	1.6	1.1	3.1	1.8	0.7	2.4	3.6	1.6	1.7	1.5	2.4
FOOD PREPARATION TYPES [*N* (%) OR %]															
Exclusive use of ready-prepared baby foods	396 (13.2)	22.5	21.2	20.9	16.5	19.3	18.5	19.6	14.6	13.5	5.4	5.5	3.4	2.0	0.8
Use of both ready-prepared baby foods and homemade foods	1,588 (53.0)	54.2	58.0	62.1	61.3	65.8	59.5	58.9	67.2	58.1	50.5	50.8	37.1	26.1	14.4
Exclusive use of homemade food and/or non-specific foods	1,015 (33.8)	23.3	20.8	17.0	22.2	15.0	22.0	21.4	18.2	28.4	44.1	43.7	59.6	71.9	84.8
FEELINGS REGARDING THE INTRODUCTION OF SOLIDS [*N* (%) OR %]															
Unconcerned	1,225 (40.9)	37.3	41.0	43.4	42.8	41.1	34.4	36.9	35.7	38.1	42.6	40.2	43.3	43.8	53.6
Eager	793 (26.4)	50.0	41.3	36.6	36.6	20.9	25.6	28.0	25.6	19.5	16.1	20.9	20.2	13.8	20.0
Reluctant	981 (32.7)	12.7	17.7	20.0	20.6	38.0	40.0	35.1	38.7	42.4	41.2	39.0	36.5	42.4	26.4

CF, complementary feeding; BLW, baby-led weaning.

**Dichotomous variables; Only the "yes" modality is presented; for the sex variable, only the girl modality is presented.*

[a]*For some age classes and some variables, information was not reported, and the total number of responses is not always equal to 2,999.*

significant differences between 10-month-old children and those aged between 25 and 36 months]. For the more complex textures (T3 level), the acceptance vs. offered ratio steadily increased with age [$F_{(10,2153)} = 29.4$, $p < 0.001$], and the age classes between 8 and 11 months had a significantly lower proportion of accepted foods (47–62%) than the older age classes (>70%). Within the older age classes, T3 level acceptance significantly increased between 12–13/15 months (∼71%) and 25/29–30/36 months (∼80%).

The increase in texture acceptance with age was associated with a decrease in the difficulties in eating ($p < 0.05$ for all texture levels). The ratio of foods directly spat out vs. food texture combinations offered also decreased with age ($p < 0.05$ for all texture levels) and concerned relatively few foods in all age classes: 2.5% of the T1 foods, 4.5% of the T2 foods, and 10% of the T3 foods. At all age classes, the ratio of food eaten without difficulty vs. foods offered ranked in the following order: T1 > T2 > T3. These differences were significant ($p < 0.001$), except for the age classes 7, 8, and 9 months, where the ratios for T1 and T2 were not significantly different.

The Texture Acceptance Score (TextAcc) as a Function of Age

The distribution of the TextAcc over age classes is presented in **Figure 3**. As expected, TextAcc significantly increased with age [$F_{(13,2985)} = 381.7$, $p < 0.0001$]. The score increased steadily between 4–5 and 16–18 months (1.7 ± 0.7 to 3.6 ± 0.7, respectively) and then more slowly up to 30–36 months (4.1 ± 0.6) (**Figure 3**). This shows that new food texture combinations become accepted throughout the entire CF period. The high variability of this score raises the question of its main predictors.

Factors of Food Texture Acceptance

Among the 17 variables initially associated with TextAcc (**Supplementary Material 3**), eight were still significant when assessed in multivariate analysis corrected for weight for length z-score (**Table 2**). TexAcc was better explained for children in the age classes between 9 and 16–18 months, as observed from the R^2 (**Table 2**), than for those of earlier or later ages.

At most age classes, TexAcc was related to the numbers of food-texture combinations offered (T1, T2, and T3; **Table 2**). The direction of the association depended on the texture level considered. TextAcc was positively associated with the number of T2 foods introduced in the age class between 6 and 16–18 months (except at 12 months, $p = 0.32$) and with the number of T3 foods for the classes from 6 months up to 25–29 months. TextAcc was negatively associated with the number of T1 foods introduced in the age classes between 9 and 30–36 months [except for the 22–24 months class ($p = 0.12$)]. In other words, the less children were exposed to purees and the more they were exposed to pieces (soft or hard), the higher their acceptance score.

Other factors that were associated with TextAcc included some children's developmental characteristics. The TextAcc score was mainly related to the ability to eat with fingers. Among 11-, 12-, 13–15-, 16–18-, and 19–21-month-old children, those who never/rarely ate with their fingers had lower acceptance scores than those doing so more frequently (**Table 2**). Surprisingly, a

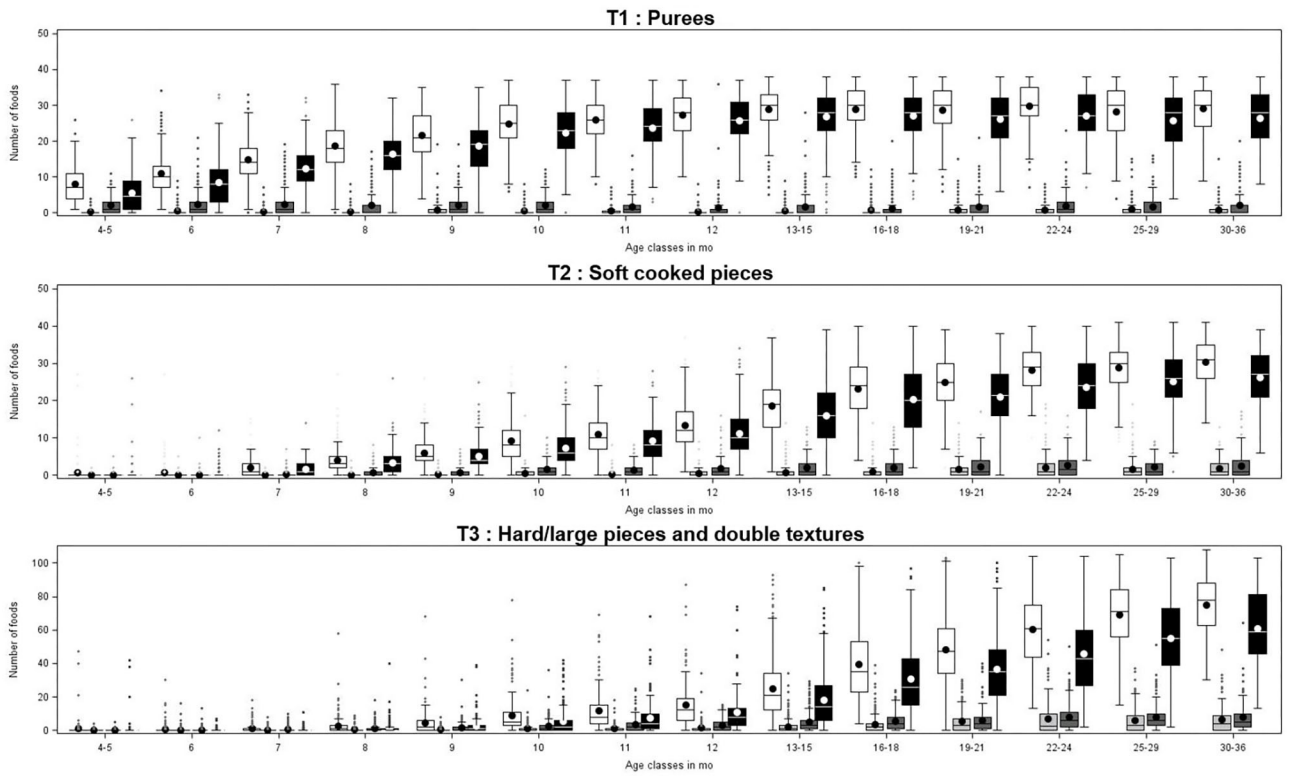

FIGURE 1 | Mean number of food-texture combinations offered (white box) and their level of acceptance (spat out: light gray box, eaten with difficulties: dark gray box, and eaten without difficulty: black box) as a function of children's age class and texture levels (T1, T2, and T3).

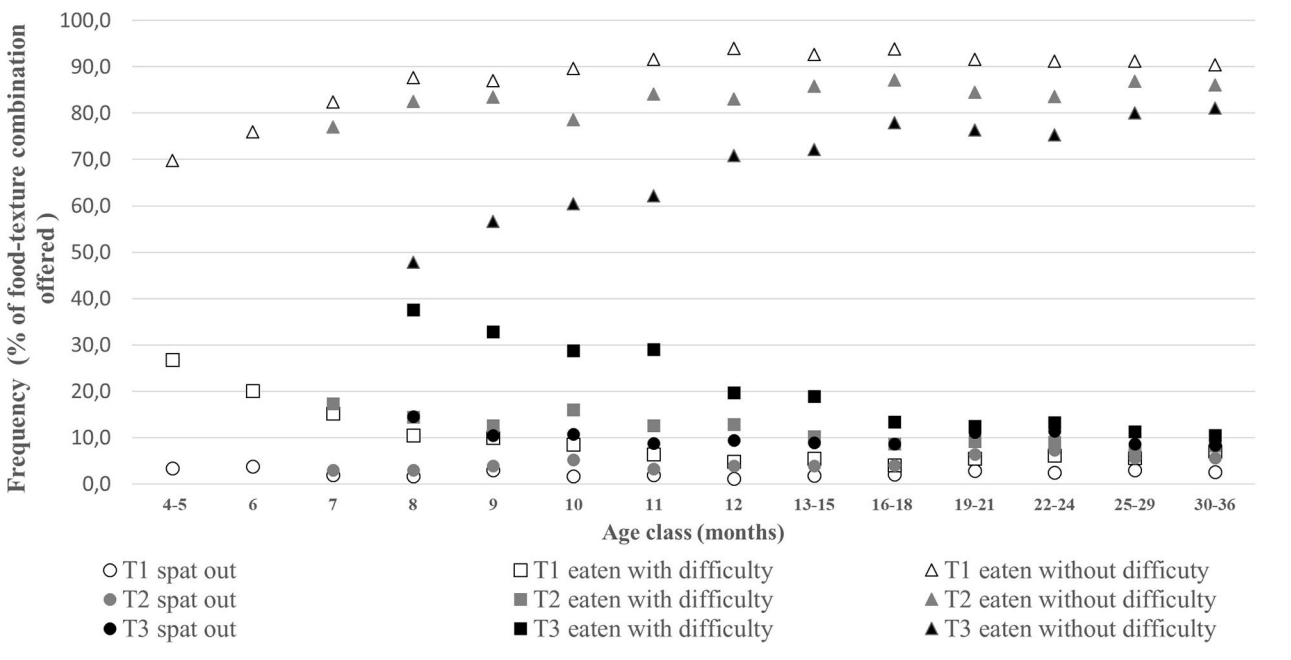

FIGURE 2 | Mean percentages of the number of combinations "spat-out," "eaten with difficulties," and "eaten without difficulty" over the number of combinations offered, by texture level and by age class. Frequencies are presented when the median of offered combination(s) was at least equal to 1 (T2 and T3 data are thus missing up to 7 and 8 months, respectively).

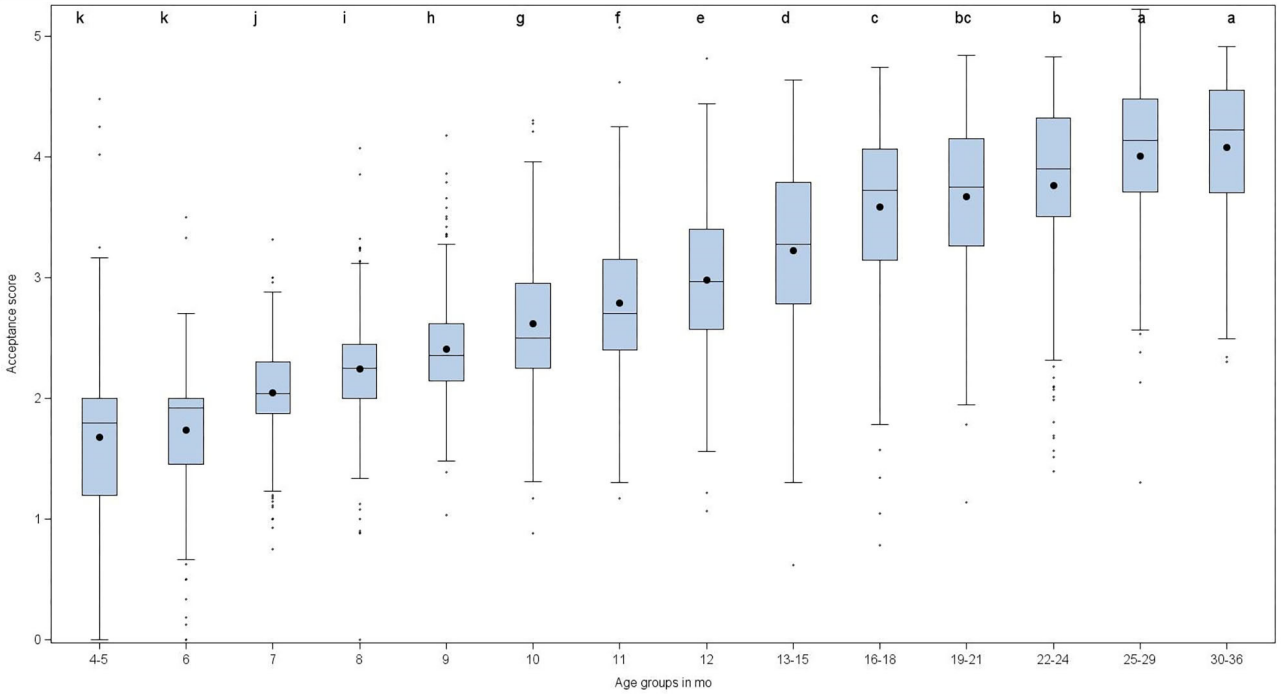

FIGURE 3 | Texture acceptance score (TextAcc) per age class. Mean scores associated with different letters (a, b, …, k) are significantly different ($p < 0.05$).

significant opposite effect was observed at 7 months, an age period when food pieces (T2 and T3) were barely introduced. TextAcc was also related to gagging (**Table 2**). In the following age classes, 6, 11, 12, 13–15, 16–18, 19–21, and 25–29 months, children reported to rarely or often gag had a lower TextAcc score than those for whom this behavior was never observed (**Table 2**). The number of teeth was associated with a higher TextAcc score for the group of 4-5-, 10-, and 13–15-month-old children.

Concerning feeding practices, 6- and 12-month-old children introduced earlier to CF had a higher texture acceptance score. Finally, the feeling reported by mothers concerning the introduction of solids was significantly associated with TextAcc. For 7, 9, 11, 13–15, and 30–36-month-old children, the children of mothers who reported themselves as being reluctant to introduce solids had a lower texture acceptance score than those of mothers who were unconcerned.

DISCUSSION

This study aimed to evaluate parental self-reports of children's ability to eat foods of different textures and to determine factors associated with children's texture acceptance as a function of age. Texture Acceptance (proportion of foods texture-combination easily eaten over the total introduced) increased from the beginning of CF until the end of the 3rd year of life and decreased with texture level (purees > soft and small pieces> big/hard pieces and double texture) at each age studied. Associated factors were related to specific aspects of parental feeding practices and feelings concerning food piece introduction and some developmental characteristics of children.

Food Texture Acceptance: Evolution With Age

Patterns of texture acceptance (i.e., ability to eat without difficulty) were closely related to the patterns of food offering, suggesting that when parents offered solid foods with a specific texture to their child, these foods or textures generally became accepted without difficulty. This could be explained by the fact that food textures are introduced in the diet in a period when children have already acquired the necessary skills to eat them or can easily develop them upon exposure to textures. This is in agreement with the previous observation that non-pureed food-texture combinations (T2 and T3) were introduced rather late to children in France [see also (31)] and that children were able to handle textures in small quantities at an earlier age than their parents' feeding practices (14).

Acceptance developed mainly between the start of CF up to 7 months for pureed foods, which is in agreement with the acceptance frequency for smooth and rough purees observed at 6 months (14). Acceptance for small and soft pieces and after that, more challenging textures (T3 levels) develop up to 30–36 months in our study. The increase in acceptance is related to the transition from sucking to chewing [∼8–10 months, (14)], the development of chewing skills for textured foods during the CF period, as observed earlier from the number of chews required to swallow foods (4) and the ability to form particles from a

TABLE 2 | Associations between food texture acceptance score (TextAcc) and children's characteristics and skills and maternal feeding practices from multiple linear regression models performed by age class [the reported figures are beta values (95% confidence intervals)].

Age groups (in months)	Tested variable modality	4-5	6	7	8	9	10	11	12
N		**142**	**283**	**235**	**243**	**187**	**195**	**168**	**137**
***N* observations used**		**128**	**269**	**222**	**231**	**174**	**190**	**156**	**127**
EXPOSURE TO FOOD TEXTURE									
T1		**0.03***	0.00	−0.00	−0.01	**−0.02*****	**−0.02*****	**−0.01****	**−0.02****
		(0.00, 0.05)	(−0.01, 0.02)	(−0.02, 0.01)	(−0.02, 0.00)	**(−0.03, −0.01)**	**(−0.03, −0.01)**	**(−0.03, −0.06)**	**(−0.04, −0.01)**
T2		–	**0.08****	**0.07*****	**0.05 *** **	**0.06*****	**0.04*****	**0.04*****	0.01
			(0.03, 0.14)	**(0.04, 0.10)**	**(0.02, 0.07)**	**(0.04, 0.08)**	**(0.02,0.05)**	**(0.02, 0.05)**	(−0.01, 0.03)
T3		–	**0.04***	**0.03***	**0.03*****	**0.02****	**0.02*****	**0.02*****	**0.02*****
			(0.01, 0.08)	**(0.01, 0.06)**	**(0.01, 0.04)**	**(0.01, 0.03)**	**(0.02, 0.03)**	**(0.01, 0.03)**	**(0.01, 0.03)**
CHILDREN CHARACTERISTICS AND SKILLS (MODALITY OF REFERENCE)									
Number of teeth		**0.16*****	0.02	−0.01	0.00	0.02	**0.03***	0.02	0.03
		(0.09; 0.23)	(−0.06, 0.10)	(−0.04, 0.03)	(−0.03, 0.03)	(−0.00,0.46)	**(0.00, 0.05)**	(−0;01, 0.05)	(−0.05, 0.06)
Current weight–for–length z–score		0.00	−0.02	0.04	**0.04***	0.03	0.00	−0.00	0.05
		(−0.07, 0.08)	(−0.06,0.03)	(−0.01, 0.08)	**(0.00, 0.08)**	(−0.01, 0;07)	(−0.05, 0.06)	(−0.06, 0.05)	(−0.01, 0.11)
Eating with fingers (sometimes/often)	Rarely/never	−0.05	−0.13	**0.25****	−0.07	−0.06	−0.12	**−0.21*****	**−0.24***
		(−0.49, 0.39)	(−0.36,0.11)	**(0.07, 0.42)**	(−0.21, 0.07)	(−0.19, 0.06)	(−0.25, 0.01)	**(−0.34, −0.09)**	**(−0.45, −0.04)**
Gagging frequency (never)	Rarely	−0.19	**−0.16***	−0.02	−0.05	−0.06	−0.02	**−0.16***	−0.07
		(−0.43, 0.13)	**(−0.30, −0.02)**	(−0.14, 0.10)	(−0.17, 0.07)	(−0.18, 0.06)	(−0.15, 0.12)	**(−0.29, −0.01)**	(−0.27, 0.12)
	Sometimes/often	−0.16	**−0.18***	−0.01	−0.02	−0.13	−0.14	**−0.19***	**−0.24***
		(−0.40, 0.09)	**(−0.32, −0.04)**	(−0.15, 0.12)	(−0.15,0.10)	(−0.27, 0.00)	(−0.31, 0.03)	**(−0.34, −0.03)**	**(−0.47, −0.05)**
FEEDING PRACTICES (MODALITY OF REFERENCE)									
Age of CF		−0.15	**−0.17****	−0.04	−0.05	−0.05	−0.02	−0.00	**−0.10****
		(−0.44, 0.13)	**(−0.29, −0.06)**	(−0.11, 0.03)	(−0.12,0.01)	(−0.10, 0.01)	(−0.09, 0.06)	(−0.09,0.07)	**(−0.17, −0.03)**
Feelings re: introduction of solids (unconcerned)	Eager	0.02	0.07	−0.03	0.01	−0.06	0.12	−0.10	−0.03
		(−0.20, 0;23)	(−0.05, 0.20)	(−0.15, 0.08)	(−0.10, 0.12)	(−0.21, 0.09)	(−0.04, 0.28)	(−0.25, 0.05)	(−0.28, 0.18)
	Reluctant	0.00	0.05	**−0.15***	−0.04	**−0.13***	0.03	**−0.19***	−0.00
		(−0.32, 0.32)	(−0.13, 0.22)	**(−0.29, −0.02)**	(−0.17, 0.10)	**(−0.25, −0.01)**	(−0.11,0.18)	**(−0.34, −0.03)**	(−0.20, 0.20)
Model R^2		0.31	0.20	0.29	0.36	0.52	0.52	0.62	0.48

(Continued)

TABLE 2 | Continued

Age groups (in months)	Tested variable modality	13–15	16–18	19–21	22–24	25–29	30–36
N		**370**	**279**	**254**	**178**	**203**	**125**
***N* observations used**		**351**	**255**	**226**	**150**	**163**	**88**
EXPOSURE TO FOOD TEXTURE							
T1		**−0.02*** **	**−0.04*** **	**−0.02***	−0.02	**−0.03*** **	**−0.05*** **
		(−0.03, −0.01)	**(−0.05, −0.03)**	**(−0.03, −0.00)**	(−0.05,0.01)	**(−0.05, −0.02)**	**(−0.07, −0.02)**
T2		**0.03*** **	**0.04*** **	0.01	0.01	0.00	0.02
		(0.02, 0.05)	**(0.02, 0.06)**	(−0.01, 0.02)	(−0.03, 0.04)	(−0.02, 0.03)	(−0.02, 0.07)
T3		**0.03*** **	**0.01** **	**0.01*** **	**0.01** **	**0.02*** **	0.01
		(0.02, 0.05)	**(0.00, 0.01)**	**(0.01,0.02)**	**(0.01, 0.03)**	**(0.01, 0.03)**	(−0.01, 0.02)
CHILDREN CHARACTERISTICS AND SKILLS (MODALITY OF REFERENCE)							
Number of teeth		**0.02***	0.00	0.00	−0.02	0.01	0.02
		(0.00, 0.04)	(−0.01, 0.02)	(−0.02, 0.02)	(−0.05, 0.02)	(−0.02, 0.04)	(−0.03, 0.06)
Current weight-for-length z-score		−0.02	0.04	0.04	0.00	0.02	−0.08
		(−0.07, 0.02)	(−0.01,0;08)	(−0.01; 0.10)	(−0.10, 0.10)	(−0.05, 0.10)	(−0.17, 0.01)
Eating with fingers (sometimes/often)	Rarely/never	**−0.13***	**−0.31** **	**−0.32***	−0.04	−0.02	0.15
		(−0.25, −0.01)	**(−0.49, −0.11)**	**(−0.58, −0.07)**	(−0.38, 0.30)	(−0.31, 0.28)	(−0.19, 0.48)
Gagging frequency (never)	Rarely	−0.08	−0.06	**−0.17***	0.07	−0.06	−0.26
		(−0.20, 0.02)	(−0.19, 0.08)	**(−0.32, −0.02)**	(−0.18, 0.31)	(−0.23, 0.11)	(−0.54, 0.02)
	Sometimes/often	**−0.26*** **	**−0.36*** **	−0.05	−0.27	**−0.50** **	−0.16
		(−0.40, −0.12)	**(−0.50, −0.17)**	(−0.29, 0.16)	(−0.59, 0.06)	**(−0.80, −0.20)**	(−0.49, 0.18)
FEEDING PRACTICES (MODALITY OF REFERENCE)							
Age of CF		0.00	−0.03	0.02	0.05	−0.02	0.02
		(−0.05, 0.05)	(−0.08, 0.02)	(−0.05, 0.08)	(−0.07, 0.16)	(−0.08, 0.03)	(−0.05, 0.08)
Feelings introduction of solids (unconcerned)	Eager	−0.12	0.02	−0.17	−0.10	0.16	−0.24
		(−0.24, 0.03)	(−0.16, 0.19)	(−0.36, 0.02)	(−0.42, 0.21)	(−0.07, 0.39)	(−0.54, 0.06)
	Reluctant	**−0.18** **	−0.08	−0.16	−0.26	0.06	**−0.31***
		(−0.29, −0.07)	(−0.21, 0.06)	(−0.33, 0.00)	(−0.54, 0.03)	(−0.11, 0.23)	**(−0.61, −0.02)**
Model R^2		0.59	0.56	0.34	0.27	0.48	0.37

Significant effects are highlighted in bold: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). For categorical data, the modality of reference is specified within brackets.
"–:" not tested because mostly not offered in this age class.
CF, complementary feeding.

model food gel (13). An earlier study based on a parental report conducted in the US (28) reported that children were eating food with tiny lumps without gagging at 8.7 months (age range: 4.8–15.5) and chewed softer foods at 9.4 months (6.0–14.0). At this age (∼9 months), we observed that 87% of the purees (both smooth and rough) and 83% of small/soft pieces (that can be squeezed between the tongue and palate) were eaten without difficulty. Carruth and Skinner (28) reported that children are able to chew and swallow firmer foods without choking at ∼12.2 months, although with a very large age range (7.5 and 20.0 months). In our study, acceptance for T3 texture was ∼70% at 12 months and was found to continue to develop until 30/36 months. This development is in line with the development of chewing function characterized by mandibular motor control and chewing muscle coordination (11). Despite an increase in texture acceptance with age throughout the entire CF period and a decrease in the gap between texture levels, we observed that purees were still on average better accepted (eaten without difficulty) than soft and small pieces and that hard/large pieces and double texture foods were the least easily eaten at the end of the CF period.

Acceptance and Maternal Feeding Practices

Food texture introduction was found to be the main factor associated with acceptance. In a given age group, children having the higher acceptance score were those who had been offered the opportunity to experience a large variety of foods offered as pieces or double texture (soft and small pieces until 18 months and hard/large pieces and double texture until 29 months). This is in agreement with two previous studies, which concluded that the age of introduction of lumpy foods (17, 18) and familiarity with different textures (15) are important factors for developing food acceptance. All three studies contribute to the notion that a timely and repeated introduction of a variety of textured food is needed to achieve good food acceptance.

Concerning other maternal feeding practices evaluated in the survey, children in the present study were introduced to CF (timing and type of food) in agreement with the National French guidelines (34), [see (31) for discussion on practices] and mostly via traditional spoon feeding. Some factors were initially associated with TexAcc (breastfeeding in children of 10, 12, 20/24 months, eating with the family (10 of 14 age classes), exclusive use of homemade or non-baby commercial foods (eight age classes) and exclusive use of ready-prepared baby foods (11, 13/15, and 16/18 months) but were no longer significant when assessed in multivariate analysis, suggesting that they may play an indirect role in acceptance by influencing maternal practices with regard to texture introduction.

Last, compared to mothers who were reluctant to introduce solid foods, mothers who were unconcerned about the introduction of solids had children who better accepted texture. This is partly in agreement with the earlier observation that reluctant mothers introduced less texture in their children's diet than other mothers (31). As texture introduction was taken into account in the current analysis, data suggest that maternal feelings concerning foods for their children may also affect measured acceptance *via* an additional way than limited exposure to textured foods. This way can be 2-fold: mothers reluctant to introduce solids may have underestimated their child's acceptance of texture, or they may have insisted less when proposing a food with a difficult texture during the meal. It would be interesting in future studies to better understand the reluctance of some mothers to introduce foods pieces, as this may help to find ways to improve texture acceptance in their child.

Acceptance of Food Textures and Associations With Developmental Characteristics of Children

Reported motor and feeding skills evolved with age and were congruent with the time line of typically developing children reported previously: ability to sit alone (28) and drooling frequencies (35) were in agreement with previous reports. Approximately half of infants were able to eat with their fingers at 11–12 months, which seems later than reported in a US survey where 98% of 9-11-month-old children were reported to grasp foods with their hand (36). Gagging frequency was reported sometimes/often in 20–30% of the children between 4 and 12 months, which is within the frequency range observed from video analysis of 8- to 9-month-old children eating pieces (19). Gagging and eating with fingers predicted acceptance for children in the age classes between 11 and 29 months. Children self-feeding with their fingers frequently accepted textured foods better than those doing it less often. The beginning of the 2nd year coincides with the introduction of soft and hard textures. At this stage, the child's tactile experience is stimulated at both the digital and oral levels: first, he/she holds the food with his/her hands, and then, he/she continues exploring it with the mouth (37). The present study is in agreement with others run in preschool and school children reporting that feeling the texture with their hands increased acceptance of a food with the same texture (23, 38). Gagging was associated with lower texture acceptance at the beginning of CF (6 months) and later during CF (11, 12, 13–15, 16–18, and 25–29 months). These ages correspond to the introduction of the first non-smooth purees (i.e., rough purees at 6 months) and of foods with pieces and harder texture (2nd year of life). A relationship between gagging and texture has been reported earlier in 8-month-old children: gagging in response to food pieces was more frequent than in response to pureed foods (19). Gagging is a normal reflex due to the high tactile sensitivity of the inner sides of the cheeks (39) and usually decreases upon repeated exposure to (new) textures. However, gagging has been reported earlier as behavior occurring in children presenting eating difficulties (40) and in tactile defensive children (22). Here, gagging was evaluated as a general behavior of the child and not as a specific behavior related to a given food, so unfortunately, these data do not enable a further understanding of which food texture may specifically provoke a gag response.

Texture acceptance was also initially associated with children's ability to sit alone in young children (in the age classes between 4–5 and 7 months) and with the ability to eat alone with a fork in the

older children (classes 13–15 to 24 months). However, these skills were no longer associated in the multivariate model, suggesting that they indirectly impact food texture acceptance by influencing food texture exposure.

For few age classes, texture acceptance was associated with children's dentition. The number of teeth reported by parents was in agreement with normal dental eruption (41, 42) and was significantly associated with texture acceptance for 4- to 5-, 10-, and 13- to 15-month-old children, suggesting the favorable role of incisors and first molars (which are known to erupt around this age) on children's ability to eat solids. Initial binary analyses (**Supplementary Material 3**) revealed a positive association between dentition and texture acceptance ($p < 0.05$) for six age classes (4-5, 8, and between 10 and 15 months). After multivariate analyses, this effect remained significant for only three of them, suggesting that the associations between dentition and acceptance were confounded with other factors having more impact on acceptance. Indeed, we previously reported that dentition is a predictive factor for exposure to texture: the number of teeth was considered by mothers as a signal for introducing textured food (31).

Strengths and Limitations

Using parental reports, details of children's texture acceptance have been generated during the entire CF period for a wide range of food textures and based on a relatively large number of children. Moreover, the extent to which individual characteristics, children's feeding skills and other personal characteristics as well as maternal feeding practices and feelings influence acceptance was evaluated. Our results are in agreement with previous experimental studies concerning both the development of texture acceptance and the favoring factors, which suggests that parental reports of children's eating ability are a valuable assessment that could be used in future studies. The factors associated with texture acceptance were hierarchized between those directly affecting acceptance and those influencing exposure.

However, some limitations to this work are worth mentioning. The included population is a convenient sample, which limits the generalization of the results to the national population. Primiparity and educational attainment were higher in our sample than reported in the general French population (43). These parameters are known to influence maternal feeding practices and therefore may have impacted the results. In addition, participating mothers were registered and recruited *via* the Bledina potential consumers database; however, they were not necessarily feeding their child (exclusively) with commercial baby food (only 14% of them were exclusively using this type of food to feed their child). Thus, future works should aim to extend the current study in a sample that is more representative of the national population, in terms of both parents' and children's characteristics.

A second limitation concerns the design of the questionnaire. First, in its present format, it contains 188 food-texture combinations, which is a compromise between covering a wide range of relevant foods and textures, trying to have a balanced list of foods and textures for the different food categories and being short enough for parents to not be discouraged from completing it. The results of our study suggest that the questionnaire could be simplified for future studies without losing information, as current data allowed us to identify a subset of combinations rarely offered to children regardless of age. Second, although a previous study from us revealed a good agreement between parents and experimenter evaluation when assessing acceptance based on swallowing a food in a behavioral situation [(14), data not shown], a validation of the parental responses in the survey against behavioral measures has not yet been done and cannot be made with the current data set. Ideally, a future study should evaluate the validity and reproducibility of this questionnaire. Finally, the reported relationships between some developmental characteristics of children—food exposure—food acceptance are correlational, and further research based on randomized interventions should be done to help us to better understand these relationships.

CONCLUSION

The development of food texture acceptance during the complementary feeding period and associated factors were determined from parental reports of their child's ability to eat selected foods and textures. This study confirmed that complementary feeding is an important period for children to accept new textures. Acceptance develops with age upon exposure to a variety of textures, and in each age class, it varies as a function of the texture level. Some developmental characteristics of children, maternal feeding practices and maternal feelings with regard to the introduction of solids were associated with acceptance either directly or by modulating exposure. Children's ability to eat with their fingers, frequency of gagging and, to a lesser extent, their dentition and their mothers' feelings with regard to the introduction of solids were the major predictors of acceptance. This survey gives important information on the development of acceptance for textured foods by children over the entire complementary feeding period in France. It confirmed the importance of prior exposure to a variety of textures for the acceptance of textured foods and provides evidence regarding the involvement of several personal factors.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are available by request to corresponding authors, after permission by the industrial partner. Requests to access the datasets should be directed to Carole Tournier, carole.tournier@inrae.fr.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes Est III, no. 2015-A00323-46. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CT, LD, AM, HW, and SN designed the research. LD collected the data. LD, CT, EK, and SN analyzed the data. CT and SN wrote the initial paper, which was then reviewed by LD, AM, and HW. All authors are responsible for the study findings, read, and approved the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This research was supported by Blédina SA (Blédina is a supplier of baby food products), the Research and Technology National Association (ANRT), the Conseil Régional Bourgogne, Franche-Comté (PARI grant), and the FEDER (European Funding for Regional Economic Development).

ACKNOWLEDGMENTS

The authors acknowledge the families who participated in the study and Marion Dusoulier (Blédina R&I) for her valuable contribution to the design of the study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.616484/full#supplementary-material

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Conflict of Interest: LD and AM are employees of Blédina R&I. HW has been an employee of Danone Nutricia Research.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

PERSPECTIVE
published: 09 February 2021
doi: 10.3389/fnut.2021.634410

Food Oral Processing—An Industry Perspective

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We illustrate how scientific understanding of Food Oral Processing enables food product development with specific benefits for several target populations. *in vivo*, *in vitro,* and *in silico* approaches are discussed in the context of their ability to quantify oral processing from the molecular to the macroscopic scale. Based on this understanding, food structures with enhanced performance in terms of hedonic and nutritional properties as well as appropriateness for age and certain medical conditions can be developed. We also discuss current gaps and highlight development opportunities from an industry perspective.

Keywords: biophysics, mechano-reception, taste molecular release, aroma release, sensory perception, age-appropriate products, nutrition for special medical purposes, biomimetic

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *27 November 2020*
Accepted: *15 January 2021*
Published: *09 February 2021*

Citation:
Devezeaux De Lavergne M, Young AK, Engmann J and Hartmann C (2021) Food Oral Processing—An Industry Perspective. Front. Nutr. 8:634410. doi: 10.3389/fnut.2021.634410

INTRODUCTION

Food Oral Processing as the initial phase of food breakdown in the human body is critical from both nutritional and sensorial points of view. The central part of sensory perception is formed dynamically during oral processing (1–3). Understanding this breakdown process provides opportunities for food innovators to offer new sensory experiences to consumers (4). Furthermore, this is also the phase where the food is transformed into a swallowable and digestible bolus. The ability to break down food is a skill acquired in early childhood (5) which can be compromised under certain medical conditions such as dysphagia (6) at a later stage in life (**Figure 1**).

From an industry perspective, it is thus of utmost importance to understand food oral processing along several dimensions: consumer group (across the life span), food matrices (liquid, solid, degree of structure), and targets (sensory, behavioral, nutritional etc.).

In agreement with other studies in the field, those examples reveal great potential but also limitations of this emerging discipline. One important limitation for further translation into food industry applications are constraints related to experimentation in humans (sensory tasting, sampling of saliva, bolus, clinical trials) often requiring considerable recruitment, training, and scheduling effort/cost.

Given the complexity of physical processes and kinematics in oral processing, the need for a clear purpose when designing *in vitro* or computational (*in-silico*) models should be emphasized: what is the key output (target), e.g., residence time in the mouth, maximum bolus deformation? What are key control variables (e.g., speed of jaw movement)? Which influencing factors (e.g., dimensions of oral cavity) are assumed constant and with what justification? Mere "imitation" of biological processes guided by convenience of implementation can result in non-representative or even misleading results and should be avoided.

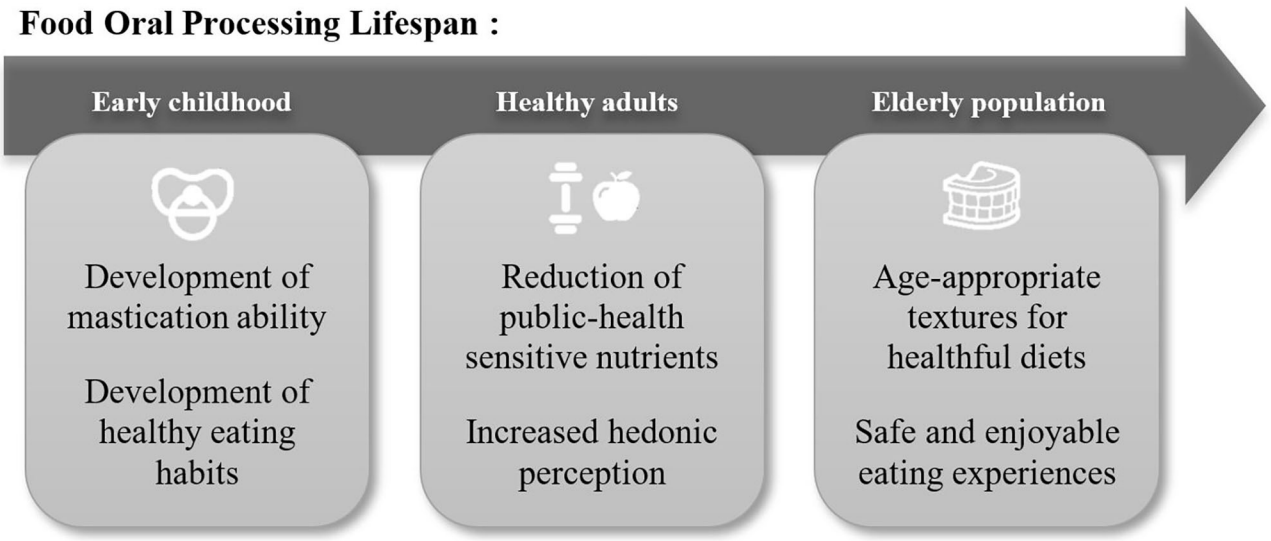

FIGURE 1 | Food oral processing lifespan.

FOOD ORAL PROCESSING TO STIMULATE AGE-APPROPRIATE EATING HABITS

At birth, food oral processing starts with the suck-swallow-breathe reflex as the only mean for nutrition at the breast or the bottle. Infant oral movements during breastfeeding have been related to weight gain and self-regulation (7, 8), better oral-facial development (9, 10) and better acceptance of food textures (11) when compared to bottle-feeding. Milk flow is one of the parameters that differs between breastfeeding and bottle-feeding, and this flow can be modeled (12). It was found that, apart from suction force, the opening size at the tip of the bottle was the main parameter impacting milk flow in the bottle. However, not much congruence between manufacturers exists in labeling of baby bottle flow (13), thus we believe regulations on nipple flow would support development of bottle feeding closer to nature.

As a child grows, the tongue protrusion reflex fades and solid foods can be introduced. The mastication process matures as the muscles, bones, teeth, lips, and tongue develop (14). It has been reported that soft solids (purées, gelatin) can be efficiently masticated from 8 months of age, while harder solids (e.g., extruded cereals) are suitable from 24 months of age (5). Simione et al. (15) studied the chewing patterns of children between 9 and 36 months of age. They found coordination and motor control improved with increasing infant age. They hypothesized that chewing developed following two broad phases: the premolar (between 9 and 18-months) and molar (between 24 and 36-months) phases. Moreover, by studying the impact of food structures on infant eating patterns, they generated data that could inform science-based recommendations regarding the safety and appropriateness of foods. Designing textures for infant food products is key to achieving successful weaning and for establishing a solid basis for healthy nutritional habits, i.e., avoiding picky eating in toddlerhood (16).

FOOD ORAL PROCESSING TO PROVIDE HEALTHY PLEASURE

Consumers demand enjoyable tastes and textures. However, hedonic appreciation is often driven by health-sensitive nutrients (including sugars, salt, saturated fats) whose intake should generally be limited. Studying Food Oral Processing helps to understand physico-chemical and physiological parameters of sensory perception of such nutrients and leads to the design of food structures that deliver high sensorial quality with minimal use of undesired ingredients.

Food structure impacts *in vivo* aroma release. For example, modulating fluid viscosity impacts the release of retro-nasal aroma (17). In addition, modulating aroma release leverages sensory cross-modal interactions and enables reductions in public-health sensitive nutrients (e.g., salt) (18). However, oral processing parameters can outweigh the impact of mechanical properties of food, i.e., during *in vivo* aroma release from cheeses (19). Macroscopic structural characteristics can also impact aroma release. For example, the compatibility of the geometry of a piece of chocolate with the oral cavity imparts distinctive in-mouth melting patterns and enhanced flavor release (4).

In parallel to aroma release, oral food breakdown impacts the release, dissolution and diffusion of tastants before they reach taste receptors. Concerning beverages, a first strategy is to impact nutrient-sensing by modulating liquid microstructure and physical properties, such as low shear viscosity modulation (20) or use of emulsion droplets as a filler (21). A second strategy to lower nutrient concentration is heterogeneous distribution.

In liquids, taste enhancement by pulsatile stimulation of taste receptors has been evidenced using gustometers (22, 23) and can be applied to products through smart packaging design (24). In solid products, macroscopic spatial distribution allowed salt and sugar reduction (25, 26).

Designing mechanical texture of foods or beverages can be as complex as designing taste and aroma since relating product mouthfeel to microstructural and rheological properties is a considerable challenge. The flows during oral sensing are complex, products evolve in the mouth, e.g., by mixing with saliva. Different sensorial pathways (gustatory, trigeminal, mechanical and even visual) all play a role in the perception of attributes like "body," "smoothness" or "creaminess" (27). In addition to refinement and extension of rheological characterization into the non-linear domain, there has been an increasing focus on behavior of thin films and "tribological" approaches (28–38). This approach has in some cases shown new correlations between *in vitro* lubrication and sensorial attributes. One should note, however, that classical tribometry yields primarily "friction factors," i.e., energy dissipation between solid surfaces lubricated by a liquid whereas "oral tribology" is also concerned with perceived "roughness," which is more related to local force fluctuations than total dissipated energy (39). Such fluctuations across oral surfaces should be considered in "oral tribology." In this context, biological roughness and compliance of specific structures (papillae) on oral surfaces play an important role in mechano-reception (40).

FOOD ORAL PROCESSING TO SUPPORT HEALTHY AGING

Above ∼45 years, nutritional needs and abilities to sense and orally process food and beverages typically evolve again (41, 42). Eating habits developed during earlier life stages should continue to be favorable for health and well-being. However, decreasing basal metabolic rates (43, 44) require lower energy intake and adaptation of eating habits (portion size, meal frequency, diet caloric density) does not occur "automatically." Specific morbidities, e.g., reduced sensation (higher perception thresholds, e.g., for sweetness), may create further challenges. Lack of specific nutrients due to lower food intake can negatively impact health and create a "vicious cycle" of lower appetite and malnutrition.

The ability to safely and efficiently prepare food for swallowing and swallow can also decline with age. Such challenges are referred to as "dysphagia." Estimates of its prevalence in specific age groups vary, but can be as high as 50% in elderly care facilities (45–47)[1]. This leads to nutritional, psychological and social burdens (48). Swallowing dysfunctions are frequently caused by neurodegenerative diseases (Alzheimer, Parkison) or severe health events such as cerebrovascular accidents ("stroke") that are more prevalent at older age. Loss of dentition also reduces the ability to masticate food and swallow effectively.

[1]Prevalence in the general population is difficult to assess due to lack of systematic screening and unified terminology.

The impact of dysphagia on dietary intake ranges from gradual (avoidance of specific foods) to severe (inability to swallow safely). In extreme cases this leaves surgical interventions or tube feeding as the sole remaining options. In less extreme cases, therapeutic interventions (training "swallowing maneuvers") and modification of *food* (softening, particle size reduction) and *beverages* (viscosity increase) enable subjects to enjoy a wide diversity of diets in terms of nutrient intake and sensorial quality. Such modifications require mechanistic understanding of oral processing and swallowing to optimally design rheological characteristics (31, 49, 50).

DISCUSSION

From this analysis it becomes obvious that thorough understanding of food oral processing leads to better products with improved benefits for consumers. However, the number and quality of studies in this area suggest that fast transfer into industry application is hard to achieve, especially given the fact that R&D capacities are very heterogeneously distributed in the food industry.

Investigation of food oral processing *in vivo* will remain crucial, as inter- and intra-individual aspects are very important for advancing understanding of food oral processing and its impact on sensory, liking, food choice, and eating habits (51, 52).

However, from an industry perspective, product design builds on food oral processing insights based on averages across a consumer group until a paradigm shift toward personalized nutrition becomes reality. Hence, we first review *in vivo* methodologies, then move on to discussing *in vitro* and *in silico* alternatives as enablers of translation into industrial practice.

Monitoring *in vivo*

Eating patterns can be measured using electromyography (EMG) and kinematics of jaw movements (KJM). For EMG, non-invasive surface electrodes can monitor the activity of superficial muscles involved in oral processing. The use of EMG for eating studies has been extensively reviewed (53–56). Of particular note is the review by Vinyard and Fiszman (57) which states that physiological research indicates EMG provides information regarding muscle activity and relative recruitment levels but cannot provide reliable estimates of absolute force and mechanical work. KJM methods consist of either a marker or transducer that is physically attached to the teeth (58, 59), or skin surface markers (or features) that track movement of the chin or other facial features (60, 61). Markers attached to the teeth have been found to significantly influence natural chewing behavior (62). The use of skin markers are less intrusive and set-up can be faster making this approach attractive for studies targeting specific consumer groups such as children or consumers with dysphagia. Simple 2D video jaw tracking of a sticker has been shown to provide similar oral processing parameter values to a 3D electromagnetic system for consumption of solid gels (61). Video recordings can generate a heavy data load that requires tedious analyses by researchers or semi-automated analyses using software but recent developments in AI are expected to alleviate this burden. Mathis et al. (63) for example, demonstrate

how pose estimation from simple markerless videography, based on transfer learning with deep neural networks, can be achieved for various body parts in multiple species across a broad collection of behaviors. Overall, using video recordings exclusively may sacrifice accuracy but due to the speed and ease of implementation (61), 2D video recordings are becoming more widely used (52).

In parallel to mastication studies, the food bolus (spit-out) can be collected allowing *ex vivo* observation of the properties of foods that have been manipulated in the mouth. A wide range of physical and chemical characterization of the food bolus can be performed, such as particle size, and mechanical properties (64). Bolus properties have been successfully linked to taste and texture perception (65–67). However, there is still a lack of alignment in methods used to characterize boluses in literature. In addition to the analysis method, oral status of participants, number of chews and food properties must be controlled to allow inter-study comparison (68).

Clinical investigation of dysphagia often uses time-resolved X-ray imaging ("videofluoroscopy") for objective and categorical characterization of swallowing (69, 70). Boluses need to contain contrast material (e.g., $BaSO_4$) to distinguish bolus and physiological structures and follow their movements. As such techniques present some exposure to ionizing radiation, they are generally restricted to subjects clinically indicated for diagnosis and are not used with healthy subjects. Recruiting sufficient eligible subjects to derive robust conclusions is hence both costly and time consuming. To avoid exposure to radiation, ultrasound can be used as a safe, non-invasive method to investigate swallowing *in vivo* (71), for instance with infants (72). Ultrasound can be used with a variety of foods (73) with minimal impact on eating patterns. However, image analysis from ultrasound measurements is laborious and there is a lack of alignment in methodology across oral behavior studies. Moreover, the low image resolution and the limited field of view does not allow a full analysis of all oral and bolus movements.

Compared to sensory, additional barriers for industry use of human food oral processing studies include: few standardized methodologies; some methodologies only allow for data collection one participant at a time; expectorated samples require immediate analysis or protocols to limit degradation during storage; separate expectorated samples are often required for each analysis type and oral processing stage of interest; more-involved data processing and analysis.

Biomimetic Devices

Major benefits of alternative approaches include product development acceleration by rapid testing of food prototypes, the obsolescence of ethical approvals, the possibility to iteratively and specifically tune experimentation toward certain aspects of investigation or to mimic different consumer groups.

Simple "oral de-structuration" steps have been carried out in literature; e.g., controlled mixing with saliva for liquids, mincing/grinding for solids (74, 75). To lubricate these *in vitro* boluses, adding sampled human saliva is the most representative solution, but a variety of artificial salivas have also been used. Enzymatic digestion of starches can be mimicked using artificial saliva containing minerals and human salivary alpha-amylase, as proposed in the international consensus for *in-vitro* digestion (76). In addition, mucins from the porcine digestive system or submaxillary bovine mucins have been used to reproduce the lubrication properties of human saliva (77). However, complex chemical interactions cannot be reproduced using artificial saliva with simplified compositions. For example, complexation with astringent compounds involves a variety of small and large salivary proteins, and these mechanisms are still yet to be fully described (78). In order to study the interaction of food compounds with the oral mucosa *in vitro* models of the salivary pellicle have been developed using human saliva (79, 80).

Artificial masticators and swallowing robots have been developed to more closely mimic Food Oral Processing (74, 81–83). Of the masticators reviewed the two most advanced devices with regards to studying oral processing are the Artificial Masticatory Advanced Machine (AM^2) and the Chewing Simulator. The AM^2 has been validated for a wider range of food types through comparisons with *in vivo* bolus particle size distribution (84–86). Whereas, the main advantage of the Chewing Simulator over the AM^2 is the on-line monitoring of volatile aromatic compound release (87–89). Currently missing from these systems are the simulation of the more complex roles of the tongue in oral processing including its interactions with products being consumed. Recent advances that could be incorporated into future systems include the development of a soft robotic tongue for studying *in vitro* swallowing systems (90) and 3D-printed soft biomimetic surfaces designed to replicate tongue topography, wettability, and tribological performance (91).

In addition to masticatory robots, swallowing robots are an emerging field of research. Reviews on such swallowing robots (92, 93) noted that despite the development of a range of devices, there is not yet one device capable of mimicking the entire deglutition process throughout the oral, pharyngeal and esophageal phases. It is important to note that these artificial masticators cannot replace human studies which are still required for: (1) system validation using particle size distribution (82, 87); and (2) identification of masticator inputs such as forces, salivary flow rate, and chewing time and frequency (89).

We expect though, that with increasing amount of data from human studies, the parametrization of such robots will be more practicable and therefore a dramatic gain in flexibility for future experiments is expected. It will thus become possible to design structures for a broader range of products with lower experimental effort and by leveraging learnings between studies more easily.

Numerical Simulation

Experimental approaches to understand food oral processing have been complemented by mathematical modeling and simulation. These approaches usually focus on an isolated aspect of food oral processing, e.g., resolution limits for detection of solid objects (94, 95), fracture mechanics [e.g., (96)], effects of friction and wear [e.g., (97)], heat transfer and melting [e.g., (4)] or swallowing (50).

Integration of numerical simulation methodologies across scales (from supra-molecular to the continuum scale) and across governing physical principles and equations (molecular dynamics, fluid dynamics, heat and mass transfer, solid mechanics) has not yet been attempted. Recent work employing Lagangrian (particle-based) models bear the potential to integrate across multiple physical phenomena (98, 99). However, further integration of multi-scale approaches is required to produce predictive *in silico* approaches that ultimately could support the food product design process.

In future research, deep learning methods may help to integrate experimental techniques, data from biomimetic devices, *in vivo* approaches and computational approaches.

Similarly to our insights on biomimetic devices, we believe that *in-silico* approaches, validated thoroughly with *in-vivo* data, and in combination with *in vitro* tools, will allow inherently coupled processes to be addressed in a single study simultaneously; for example, the quantification of structure breakdown, tastant and aroma release, mixing with saliva. This would be impossible for *in vivo* studies because of the invasiveness of the quantification methods.

WAY FORWARD

We consider the increasing demand for plant-based products (100) in various consumer groups (young adults, adults, parents, patients, seniors) as one key driver of research in the area of food oral processing as chewing abilities vary across the life span.

For plant-based meat alternatives, for example, scientific understanding for the creation of meat-like textures will determine market penetration. Yet, an additional challenge is the creation of authentic meat-like taste and aroma experiences. Scientific understanding of tastant and aroma release during the oral process is crucial, as it depends inherently on food structure, mastication performance, and thus on the trajectory of oral food breakdown from initial structure to swallowable bolus.

Many plant-based dairy alternatives exhibit dry-mouthfeel, chalkiness and astringency induced by low-molecular weight compounds but also by proteins (101). In many products these defects are currently masked through flavorings, sweeteners, fats and hydrocolloids. Nutritionally more responsible products will gain consumer acceptance only if these defects can be solved through proper understanding of food oral processing.

In this manuscript, we focus on individual products. However, we would also like to highlight recent contributions addressing food oral processing across a whole meal or even diet (102, 103). Just like heterogeneous structures in an individual product, the spatio-temporal arrangement of different meal components modulates oral processing and thus impacts hedonic (e.g., liking) or health related (e.g., intake) outcomes. As these considerations require even more complex experimental arrangements with increasing permutations of food items (products in a meal) and their textures, we expect a strong demand for more flexible and robust methods also arising from this research.

We therefore pledge for an increased research intensity toward integration of currently co-existing scientific disciplines (food science, physiology, engineering). In the short to mid-term, a portfolio of biomimetic laboratory methods mimicking food-oral-processing from micro- to macro-scale, from comminution to swallowing and from solid to liquid matrices is required to develop new food solutions. This should be backed by faster experimentation methods on humans, and complemented with further developed *in silico* approaches.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

CH initiated the work on this manuscript and brought the team of authors together. MD coordinated the writing and submission process. All authors contributed equally to the redaction and the content of this manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 12 February 2021
doi: 10.3389/fnut.2021.608095

Influence of Facial Morphology on Masticatory Function and Quality of Life in Elders Using Mandibular Overdentures: 3-Year Results

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: 18 September 2020
Accepted: 11 January 2021
Published: 12 February 2021

Citation:
Possebon APDR, Schuster AJ, Marcello-Machado RM, Martins APP, Pinto LR, Chagas-Júnior OL, Del Bel Cury AA and Faot F (2021) Influence of Facial Morphology on Masticatory Function and Quality of Life in Elders Using Mandibular Overdentures: 3-Year Results. Front. Nutr. 8:608095. doi: 10.3389/fnut.2021.608095

Background: Facial types may interfere in the oral health-related quality of life (OHRQoL) and masticatory performance of implant-retained mandibular overdenture (IMO) wearers.

Purpose: Investigate the medium-term changes in the masticatory function (MF) and OHRQoL parameters of IMO users, as a function of facial pattern, anteroposterior skeletal discrepancy, and sex.

Methods: Forty IMO users, most of them Caucasian (90%) with average age of 69.17 years were classified according to their facial pattern and antero-posterior discrepancy prior to rehabilitation. MF was evaluated by the multiple sieves method to determine the average particle size (X50), heterogeneity (B) and masticatory efficiency (ME, calculated as the percentage of material retained in the 5.6 and 2.8 mm sieves), using Masticatory performance (MP) and swallowing threshold (ST) tests. OHRQoL was measured by applying the dental impact on daily life (DIDL) questionnaire. The data were analyzed by Wilcoxon-paired tests to analyze changes in MF parameters over time, and mixed-effect multilevel regression models were employed to verify differences between groups.

Results: Significant changes were still observed in the 3rd year for the ST test with improvements in B for Mesofacial and in time for Dolichofacial individuals, while ME_2.8 deteriorated for Brachyfacial participants. B values of Class I and male individuals improved and brachyfacial individuals still presented worse homogenization (B) than Mesofacial participants in both masticatory tests. Class II and III participants still showed improvements in ME_5.6 and time compared to Class I despite increases in X50. Class II individuals needed less cycles than Class I in the 3rd year. Brachyfacial participants scored lower in the Appearance domain than Mesofacial ones in the 3rd year. Dolichofacial participants and Class III patients scored lower in the Oral Comfort domain than Mesofacial and Class I, respectively. In addition, age influenced the Pain, Oral Comfort and General Performance domains in the 3rd year.

Conclusions: Differences in facial morphology continue to influence the MF and OHRQoL outcomes in the 3rd year, and age influenced some OHRQoL domains. Brachyfacial individuals continue to benefit least from rehabilitation with IMO according to masticatory parameters.

Keywords: mandibular overdentures, facial profile, oral health-related quality of life, facial morphology, anteroposterior skeletal discrepancy, masticatory function

INTRODUCTION

The masticatory function (MF) of total edentulous individuals can be directly affected by the facial pattern (FP), by the anteroposterior discrepancy (ASD), and the type of prosthetic rehabilitation (1). According to the specialized literature, facial morphology shows cephalometric differences between ethnic groups (2–4). As an example, studies have shown that black and female individuals have a greater depth of the maxilla, whereas white individuals and men have a greater tendency for cranial deflection. Meanwhile, angular cephalometric measurements show no difference between these groups. The facial type is genetically established, and gender influences facial type, mainly through muscle pattern (3, 4). In terms of FP, Mesofacial individuals present a balanced bone profile and facial musculature, thus having a more predictable prognosis during prosthetic rehabilitation with conventional complete dentures (CCD), and consequently, they are considered the standard for comparisons (5, 6). Meanwhile, Brachyfacial individuals may present a greater bite force due to the biomechanical changes resulting from a compressed lower third of their face combined with strong muscle activity, which can contribute to a greater displacement of CCD during function (7, 8). Finally, Dolichofacial individuals present a greater bone height of the residual ridges in both jaws than Brachyfacial individuals (9), which would contribute to a greater stability of this type of rehabilitation with CCD and consequently a superior masticatory performance is expected. Thus, the bone and muscle characteristics of the different FP must be taken into account when planning treatment with complete dentures, to ensure a good prognosis of prosthetic rehabilitation in addition to ensuring the quality of MF (8, 10).

Another important factor capable of influencing the prognosis of totally edentulous rehabilitation is the anteroposterior discrepancy. In this framework, Class I individuals have balanced horizontal bone growth, resulting in have more predictable prosthetic rehabilitation than for Class II and Class III individuals, and thus are considered the standard for comparison (8, 10, 11). The mandibular protrusion of Class III individuals may result in a decreased vertical dimension of occlusion (VDO) (8), while the greater height of the residual bone crest in Class II individuals may enlarge VDO, which can inhibit reestablishment of the adequate maxillomandibular relationship (1, 7). The ASD deviations can often be compensated during prosthetic rehabilitation to improve MF. However, a previous study (9) found that Class III individuals had a reduced capacity to homogenize the bolus even after rehabilitation with new CCDs, suggesting that reestablishment of an effective masticatory pattern is extremely challenging in patients with this profile, and that they need a longer period to adapt to the prostheses.

Rehabilitation with implant mandibular overdentures (IMO) is preferable to the use of CCD, especially when patients experience difficulties adapting to CCD (12). IMO increase the retention and stability of the prostheses, and improve the comfort, speech and masticatory function of individuals, generating greater satisfaction with the treatment and an increase in self-reported oral health-related quality of life (OHRQoL) in the first 3 months of use (13). However, studies have shown that FP and ASD can affect the masticatory pattern that individuals develop even after the transition from CCD to IMO (1, 10, 11). Brachyfacial patients showed only a small short-term improvement in the test food trituration (10) and the FP no longer influenced the quality of chewing already after 1 year of IMO usage (11). Meanwhile, ASD negatively influenced the masticatory function, since Class II patients continued to present difficulties homogenizing the food compared to Class I individuals, both short term (10) and after 1 year of IMO use (11).

Presently, little is known about the medium-term influence of the facial morphology on OHRQoL of IMO users. In a clinical study with a 3-month follow-up time, the authors found that Dolichofacial individuals reported better scores in the Appearance and General Performance domains than Mesofacial individuals, while Class II Individuals reported higher Oral Comfort scores than Class I individuals (10). Despite the observed OHRQoL improvements of these individuals, the benefits of using IMO are perceived differently by the individuals in the short term. Thus, given the gap in the literature regarding the impact of factors such as facial pattern and the anteroposterior discrepancy may still have on MF and OHRQoL of IMO users, the objective of the present study was to investigate the medium-term changes in MF and OHRQoL parameters of IMO wearers as a function of FP, ASD, and sex. The null hypothesis of the study is that these parameters do not vary over time and that differences in the aspects PF, ASD, sex, and age are not able to influence MF and OHRQoL in the 3rd year of function.

MATERIALS AND METHODS

This longitudinal clinical study reports 3 year follow-up results of a previous study (10) performed with totally edentulous individuals assessed before transition from CCD to IMO and after 3 months. Initially, all volunteers were rehabilitated with

new CCD, which were made with thermo-polymerizable acrylic resin (VIPICRIL plus - VIPI®), artificial acrylic resin teeth (Trilux - VIPI®) assembled in bilateral occlusion. The new protheses were fabricated in the Complete Denture Clinic by undergraduate students under the supervision of two specialized professors (FF, LRP). Panoramic and lateral radiographs for all participants were performed on a Rotograph Apparatus Plus instrument by a single trained and calibrated technician. The facial pattern (FP) and anteroposterior skeletal discrepancy (ASD) classifications were performed with cephalometric analysis software (CefX version 4.5.10), using cephalometric tracings as described in the previous clinical study (9, 10). Thus, individuals were classified as Mesofacial, Brachyfacial or Dolichofacial through Rickets' analysis, based on 5 angles (14). The ASD classification into class I, II or III was based on 3 angles (15).

The original study recruited completely edentulous elderly participants of both sexes with good general and oral health according to the following inclusion criteria: users of conventional complete dentures with difficulties adapting to a mandibular complete denture, adequate oral hygiene, without self-reported systemic impairments, and with bone heights $\geq$ 10 mm in the anterior region of the mandible. Participants presenting serious systemic diseases that compromised bone healing were excluded, along with uncontrolled diabetes, history of radiotherapy in the head or neck region, previous history of oral implants installation, and participants who underwent treatment with bisphosphonates in the preceding 12 months. At the moment of the 3-year follow-up visit, all participants were $\geq$65 years and all prostheses were of good quality [category 0 according to the criteria of Vigild (16)].

After 3 months of the adaptation to the new CCD, two narrow diameter implants (Facility implant: 2.9 $\times$ 10 mm; Ti grade V, NeoPoros surface - Neodent®) were installed in the region between mental foramina and immediately connected to healing caps. After a 3-month osseointegration period, the healing caps were replaced by Equator attachments, and the IMO were installed. All implant surgeries were performed by a specialized surgeon (OLCJ) and the IMO were made by prosthodontists. In the previous short-term study, 56 individuals were evaluated; 42 of them (29 women and 13 men) met the inclusion criteria, signed the informed consent form, and participated in the study. Volunteers who presented decompensated diabetes, uncontrolled hypertension, hemorrhagic disorders, severe systemic diseases, compromised immune system, or a history of radiotherapy in the head or neck region were excluded. The participants in the aforementioned study had an average age of 66.31 years, an average time since mandibular edentulism of 24.14 years. Most individuals are Caucasian/white (90%), 1 is of Asian origin (2.5%) and 3 are brown/black (7.5%). The sample comprised 33% Dolichofacial (8 women and 6 men), 31% Brachyfacial (9 women and 4 men), and 36% Mesofacial participants (12 women and 3 men). In terms of ASD, the sample consisted of 26% of Class I (6 women and 5 men), 29% Class II (7 women and 5 men) and 45% Class III participants (16 women and 3 men). This report follows the STROBE guidelines (17), was conducted in accordance with the Declaration of Helsinki 2008, and was approved by the Research Ethics Committee of the Faculty of Dentistry UFPel, protocol (No. 69/2013). The 42 volunteers were contacted via telephone for annual assessments 1–3 years after occlusal IMO loading for evaluation of masticatory function and oral health-related quality of life (OHRQoL) would be carried out.

To assess masticatory performance (MP), individuals were instructed to chew 17 cubes with sides of 5.6 mm ($\approx$3.7 g) of "Optocal" test material for 40 cycles (18). During the swallowing threshold (ST) test, participants chewed another 17 cubes until they felt like swallowing, and the number of cycles and the time to execute the cycles were recorded (19). After both tests, the crushed material was expelled on a paper filter, dried at room temperature for 7 days and passed through multiple sieves. The material retained in each sieve was then weighed, and the average sieve opening through which 50% of the masticated material would pass (X50) and the homogeneity of the chewed particle distribution (B) were calculated. The masticatory efficiency parameters (ME_5.6 and ME_2.8) were calculated as the percentage of material retained in the 5.6 and 2.8 mm sieves (20, 21).

The OHRQoL was assessed through the DIDL questionnaire that assesses self-reported satisfaction through 36 questions divided into 5 domains: Appearance, Pain, Oral Comfort, General Performance, and Chewing (22, 23). The possible answers are agreed, neutral or disagreed, scored as +1, 0, and −1, respectively. All annual evaluations were performed by a single evaluator. Multilevel mixed effect regression models were used to estimate the effect of time on masticatory outcomes (MP, ST, and ME) and OHRQoL according to FP, ASD, sex and age, using Mesofacial and Class I patients as the reference groups. Regression coefficients and 95% confidence intervals were estimated, and p-values ≤ 0.05 were considered statistically significant. Intra-group changes in the masticatory parameters between the evaluation periods, as indicated by a significant time effect in the regression analysis, were assessed through the Wilcoxon-paired test using Bonferroni correction of the P-values (P-value required for significance $= 0.05/3 = 0.0166$). For the OHRQoL analyses, the effect size (ES) was calculated as the difference in the mean scores of the DIDL domains divided by the standard deviation of the previous evaluation period. The effect size was classified as small (ES < 0.5), moderate ($0.5 <$ ES < 0.8) or large (ES ≥ 0.8) (24). All analyses were performed using the Stata 14.1 software (StataCorp).

RESULTS

Of the 42 individuals included in the initial study, 40 returned for evaluation at 1 and 3 years. The two follow-up losses were 2 women (1 Brachyfacial and Class III, and 1 Dolichofacial and Class I) and occurred due to loss of contact between 3 months and 1 year. The average age of the individuals evaluated in this period was 69.17 $\pm$ 3.93 years.

The mixed-effect multilevel regression models showed significant differences in B values between Brachyfacial and Mesofacial individuals in the 3rd year, both in the MP ($p \leq$

TABLE 1 | Mixed-effects regression model of the masticatory performance outcomes (MP- 40 cycles) according to facial pattern (FP), anteroposterior skeletal discrepancy (ASD), sex, and age.

	Masticatory performance test			
Outcomes	**MP_X50, coefficient (95%CI)**	**MP_B, coefficient (95% CI)**	**MP_ME _5.6, coefficient (95% CI)**	**MP_ME_ 2.8, coefficient (95% CI)**
Time				
3 months	Ref.	Ref.	Ref.	Ref.
1 year	**0.51 (0.14; 0.89)**	0.55 (−0.18; 1.28)	0.28 (−0.11; 0.69)	**0.46 (0.16; 0.76)**
3 years	0.27 (−0.07; 0.62)	0.36 (−0.34; 1.07)	**0.53 (0.12; 0.94)**	0.31 (−0.04; 0.67)
1–3 years	**0.58 (0.33; 0.82)**	**0.65 (0.42; 0.89)**	**0.66 (0.40; 0.92)**	**0.58 (0.22; 0.93)**
FP				
Mesofacial	Ref.	Ref.	Ref.	Ref.
Brachyfacial	0.58 (−0.72; 1.88)	**0.25 (0.04; 0.45)**	0.49 (−0.24; 1.22)	0.60 (−0.27; 1.48)
Dolichofacial	0.45 (−0.84; 1.79)	0.28 (−0.95; 1.52)	1.06 (−0.61; 2.73)	0.93 (−0.74; 2.60)
ASD				
Class I	Ref.	Ref.	Ref.	Ref.
Class II	0.80 (−1.10; 2.70)	0.02 (−2.02; 2.08)	1.44 (−3.04; 5.94)	1.42 (−0.75; 3.60)
Class III	0.11 (−0.79; 1.01)	−0.15 (−0.59; 0.27)	−0.00 (−1.30; 1.29)	−0.26 (−0.97; 0.44)
Sex				
Male	Ref.	Ref.	Ref.	Ref.
Female	0.15 (−0.29; 0.61)	−0.17 (−0.43; 0.07)	0.01 (−0.29; 0.31)	0.08 (−0.47; 0.65)
Age (years)	−0.01 (−0.05; 0.04)	−0.01 (−0.09; 0.06)	0.22 (−0.74; 1.19)	0.06 (−0.34; 0.48)

MP_X50, particles trituration; MPB, chewing homogenization; MP_ME_5.6, % material retained in the 5.6 mm sieve; MP_ME_2.8, % material retained in the 2.8 mm sieve. Bold font indicates statistically significant differences.

0.01, **Table 1**) and in the ST test ($p \leq 0.01$, **Table 2**). Brachyfacial individuals showed worse food homogenization, as indicated by B values that are 28.78% higher in the ST test and 39.23% higher the MP test. The ST outcomes of Class II and Class III individuals also differed from those of Class I individuals in the third year, with X50 values that were 3.03 and 13.37% higher for Class II and Class III individuals, respectively ($p \leq 0.01$; $p \leq 0.01$, respectively), and 48 and 2.49% higher ME_5.6 values for Class II and Class III individuals ($p = 0.04$; $p = 0.03$, respectively). In addition, the cycle time in Class II and Class III individuals was also 14.74 and 2.47% lower than for Class I individuals ($p = 0.02$; $p = 0.04$, respectively). Meanwhile, significant differences in the number of cycles were only found between Class II and Class I individuals ($p \leq 0.01$), with a 6.09% reduction in the number of cycles at the end of the 3rd year.

Table 3 lists the coefficients and confidence intervals obtained for OHRQoL domain scores and shows that Brachyfacial and Mesofacial individuals reported differences in the Appearance domain ($p \leq 0.01$). Dolichofacial and Mesofacial individuals experienced different Oral Comfort ($p \leq 0.01$), while Class III and Class I individuals experienced a reduction in this same domain ($p \leq 0.01$). After the 3rd year, age resulted in differences in the Pain ($p \leq 0.01$), Oral Confort ($p \leq 0.01$) and General Performance ($p \leq 0.01$). **Figures 1**–**3** illustrate the changes in DIDL scores over time within each group. The Pain domain scores of dolichofacial individuals reduced by 12.50% between 3 months and year 1 (ES 0.8). For Mesofacial individuals, there was a 4.04% reduction in the average General Performance domain score between 3 months and 3 years and between 1 and 3 years (ES 0.9 and ES 1.2, respectively). Finally, Class III individuals reported a 6.06% reduction in the General Performance domain score between 1 and 3 years old (ES 2.2). Finally, women reported a reduction of 14.58% in the Appearance domain between 3 months and 3 years (ES 1.05), while their General Performance (ES 2.06) and Eating and Chewing (ES 2.20) domain scores reduced by 5.10 and 7.07%, respectively, between 1 and 3 years.

The masticatory outcomes at all evaluation periods are listed in the **Tables 4**, **5** and show that significant differences were observed only for the swallowing threshold tests. **Table 5** shows that the ST_X50 values only reduced significantly in male individuals between 3 months and 1 year by 7.83% ($p \leq 0.01$).

DISCUSSION

In our study population, a robust regression analysis indicated important changes in the mean values of the MF and OHRQoL variables over the 3-year follow-up period. Brachyfacial individuals continue to show worse food homogenization than Meso- and Dolichofacial individuals in year 3 for both masticatory function tests. Meanwhile, food trituration abilities of Class II and Class III individuals deteriorated slightly, while their masticatory efficiency (ME_5.6) improved and Class II individuals needed fewer masticatory cycles after 3 years. The improvements in masticatory efficiency are clinically insignificant for Class III individuals, but fairly large for Class II individuals. Finally, the various OHRQoL, domains continue to be influenced by both FP, ASD and age, in the same period.

TABLE 2 | Mixed-effects regression model of the swallowing threshold outcomes (ST- no predefined number of cycles) according to facial pattern (FP), anteroposterior skeletal discrepancy (ASD), sex, and age.

	Swallowing Threshold test					
Outcomes	**ST_X50 coefficient (95%CI)**	**ST_B coefficient (95% CI)**	**ST_ME_5.6 coefficient (95% CI)**	**ST_ME_2.8 coefficient (95% CI)**	**Cycles coefficient (95% CI)**	**Time coefficient (95% CI)**
Time						
3 months	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
1 year	**0.69 (0.40; 0.98)**	0.07 (−0.75; 0.91)	**0.65 (0.39; 0.92)**	**0.46 (0.22; 0.71)**	**0.61 (0.28; 0.95)**	**0.56 (0.31; 0.82)**
3 years	−0.13 (−0.46; 0.19)	0.07 (−0.09; 0.25)	−0.20 (−0.54; 0.13)	0.15 (−0.11; 0.42)	0.24 (−0.13; 0.62)	0.15 (−0.18; 0.48)
1–3 years	**0.91 (0.68; 1.13)**	**0.15 (0.10; 0.20)**	**0.94 (0.66; 1.23)**	**0.60 (0.29; 0.91)**	**0.56 (0.22; 0.89)**	**0.57 (0.18; 0.97)**
FP						
Mesofacial	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Brachyfacial	−0.01 (−0.76; 0.73)	**0.04 (0.03; 0.06)**	−0.20 (−0.87; 0.47)	0.57 (−0.20; 1.35)	−0.04 (−0.78; 0.69)	−0.03 (−0.32; 0.26)
Dolichofacial	−0.04 (−1.98; 1.90)	−0.29 (−0.98; 0.40)	0.25 (−2.82; 3.34)	0.69 (−1.18; 2.56)	−0.06 (−1.16; 1.04)	−0.02 (−0.38; 0.32)
ASD						
Class I	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Class II	**1.17 (0.40; 1.93)**	1.59 (−0.01; 3.20)	**2.97 (0.08; 5.86)**	0.92 (−0.31; 2.17)	**−1.79 (−3.27; −0.32)**	**−0.75 (−1.39; −0.11)**
Class III	**−0.59 (−0.86; −0.32)**	0.02 (−0.01; 0.07)	**−0.60 (−1.17; −0.04)**	−0.04 (−0.72; 0.63)	1.25 (−0.35; 2.88)	**1.21 (0.04; 2.39)**
Sex						
Male	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Female	−0.15 (−0.49; 0.19)	−0.04 (−0.22; 0.14)	−0.10 (−0.22; 0.01)	−0.01 (−0.31; 0.27)	0.04 (−0.84; 0.93)	0.04 (−0.52; 0.61)
Age (years)	−0.00 (−0.05; 0.04)	−0.13 (−0.52; 0.24)	0.22 (−0.63; 1.07)	0.02 (−0.45; 0.50)	0.82 (−0.26; 1.91)	0.67 (−0.23; 1.58)

MP_X50, particles trituration; MPB, chewing homogenization; MP_ME_5.6, % material retained in the 5.6 mm sieve; MP_ME_2.8, % material retained in the 2.8 mm sieve.
Bold font indicates statistically significant differences.

TABLE 3 | Mixed-effects regression model of DIDL domain scores according to facial pattern (FP), anteroposterior skeletal discrepancy (ASD), sex, and age.

	DIDL				
	Appearance, coefficient (95%CI)	**Pain, coefficient (95%CI)**	**Oral comfort, coefficient (95%CI)**	**General performance, coefficient (95%CI)**	**Eating and chewing, coefficient (95%CI)**
Time					
3 months	Ref.	Ref.	Ref.	Ref.	Ref.
1 year	*	−0.26 (−0.67; 0.14)	0.20 (−0.11; 0.52)	**1.61 (0.99; 2.22)**	**0.84 (0.63; 1.06)**
3 years	−0.26 (−0.16; 0.11)	**1.63 (0.89; 2.37)**	0.27 (−0.17; 0.73)	−0.12 (−0.38; 0.14)	0.21 (−0.07; 0.49)
1–3 years	*	**0.77 (0.24; 1.31)**	0.14 (−0.31; 0.61)	0.01 (−0.12; 0.16)	**0.78 (0.43; 1.14)**
FP					
Mesofacial	Ref.	Ref.	Ref.	Ref.	Ref.
Brachyfacial	**0.49 (0.40; 0.59)**	−0.40 (−1.85; 1.05)	0.05 (−0.57; 0.62)	−0.55 (−1.79; 0.69)	−0.16 (−0.85; 0.52)
Dolichofacial	0.03 (−0.76; 0.15)	2.59 (−1.87; 1.87)	**−0.77 (−1.12; 0.68)**	−0.65 (−2.55; 1.24)	−0.14 (−0.74; 0.45)
ASD					
Class I	Ref.	Ref.	Ref.	Ref.	Ref.
Class II	−0.05 (−0.22; 0.11)	*	0.08 (−0.10; 0.27)	*	*
Class III	−0.92 (−3.62; 1.76)	−0.10 (−0.49; 0.29)	**0.49 (0.26; 0.72)**	*	*
Sex					
Male	Ref.	Ref.	Ref.	Ref.	Ref.
Female	0.02 (−0.14; 0.19)	−0.06 (−0.38; 0.26)	−0.00 (−0.54; 0.53)	−0.05 (−0.21; 0.11)	−0.32 (−1.40; 0.77)
Age (years)	0.00 (−0.014; 0.023)	**0.00 (0.00; 0.01)**	**0.01 (0.00; 0.02)**	**0.00 (0.00; 0.01)**	0.01 (−0.00; 0.02)

*Bold font indicates statistically significant differences. *Variables show collinearity: constant variables.*

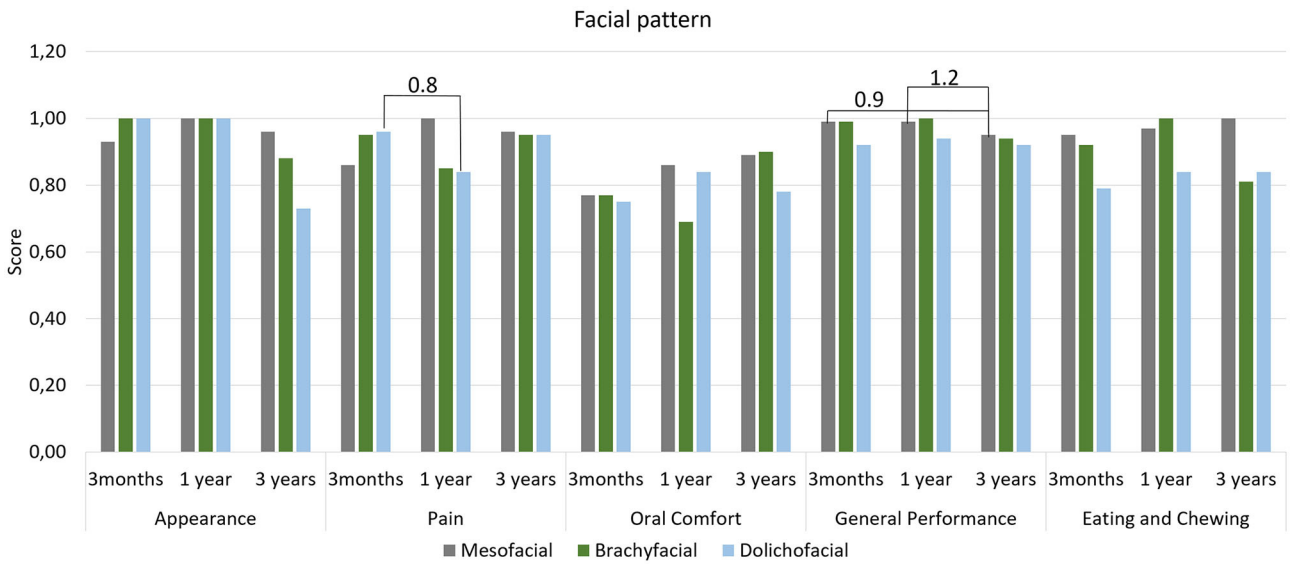

FIGURE 1 | Mean scores of DIDL questionnaire domains according to facial pattern. Periods with large effect size are indicated.

Mesofacial individuals showed improvements over time after transition to IMO, as indicated by a reduction in their B values between the 1st and the 3rd year that reflects an improvement in particle homogenization. This continuous improvement can be explained by the vertical growth (1) and balanced facial musculature activity, added to the continued long-term improvement in retention and stability promoted by IMO. Conversely, Brachyfacial individuals showed changes in the monitored parameters both over time and in relation to Mesofacial individuals. The ME_2.8 percentage in these individuals reduced by 20.84% between 1st and the 3rd year of function from 20.63 to 16.33%, showing that the medium-term use of IMO did not improve the fine particle trituration capacity of brachyfacial individuals. This worsening of the trituration

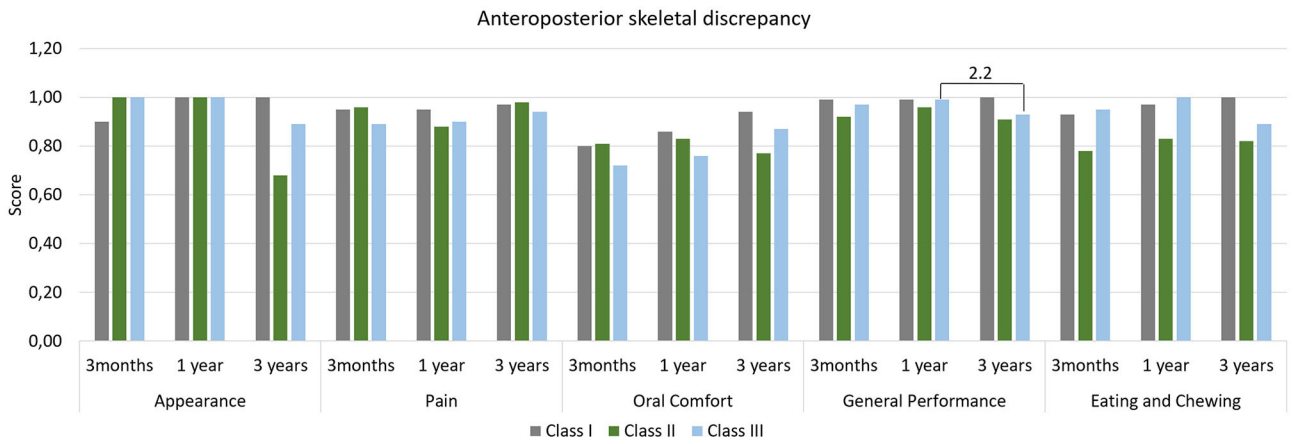

FIGURE 2 | Mean scores of DIDL questionnaire domains according to anteroposterior skeletal discrepancy. Periods with large effect size are indicated.

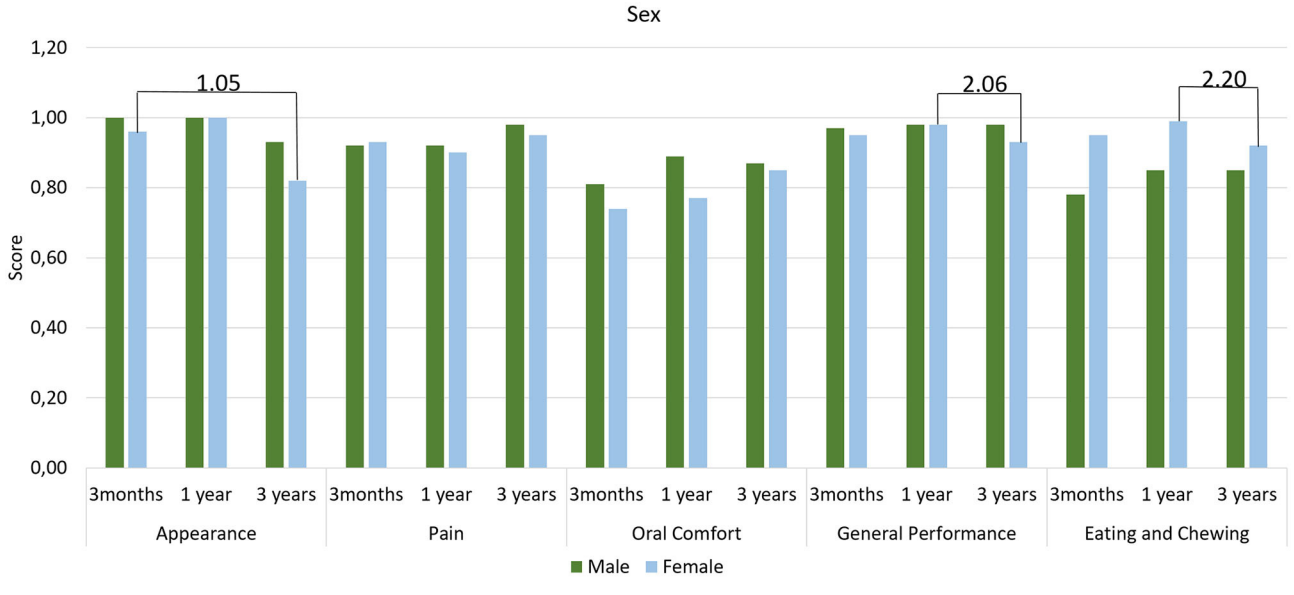

FIGURE 3 | Mean scores of DIDL questionnaire domains according to sex. Periods with large effect size are indicated.

ability can be attributed to reduced growth and height of the lower third of the face (8), which lowers the amplitude of mandibular movement during chewing, resulting in reduced mobility of the bolus in the oral cavity. Thus, the mandibular kinematics of edentulous brachyfacial individuals may have contributed to a smaller number of chewed particles that reached the 2.8 mm sieve. In this sense, these results support the idea that a larger intra-oral space favors more efficient breakdown of food particles during chewing (10).

In addition, Brachyfacial individuals also obtained less homogenous food boluses than Mesofacial individuals in the third year for both masticatory tests. This shows that the rehabilitation with IMO did not promote the expected improvement of all outcomes related to masticatory capacity, the most sensitive being the particle homogenization, followed by ME_2.8, as both outcomes started to worsen in the third year compared with the reference group (Mesofacial individuals). For Dolichofacial individuals, the masticatory cycles needed to complete the ST test reduced over time, which can be explained by the greater intraoral space that facilitates handling the food bolus and pulverization of the particles, reducing the time needed to perform the masticatory cycles (10).

In terms of ASD, only Class I individuals still showed changes in some masticatory variables in the 3rd year, as their particle homogenization capabilities improved between the 1st and 3rd year. As the homogenization capabilities of Mesofacial individuals also improved, it seems likely that the improved retention and stability provided by IMO, added to the fact that these patients feel safer when chewing, are factors that contribute to medium-term improvements in particle homogenization

TABLE 4 | Masticatory Performance (MP) outcomes (mean ± standard deviation) over time according to facial pattern, anteroposterior skeletal discrepancy and sex (Wilcoxon-paired test).

		Facial pattern			Anteroposterior skeletal discrepancy			Sex	
		Mesofacial	Brachyfacial	Dolichofacial	Class I	Class II	Class III	Male	Female
MP_X50: (mm)	3 months	4.23 (1.10)	4.78 (1.44)	4.17 (1.26)	4.28 (1.15)	4.20 (1.28)	4.55 (1.36)	3.62 (1.18)	4.72 (1.17)
	1 year	3.94 (1.00)	4.73 (1.22)	3.94 (1.01)	4.42 (1.18)	3.84 (0.96)	4.29 (1.17)	3.54 (0.92)	4.46 (1.08)
	3 years	3.43(1.24)	4.64 (1.15)	3.99 (0.70)	3.92 (1.37)	3.90 (0.83)	4.04 (1.23)	3.72 (0.91)	4.08 (1.23)
MP_B	3 months	3.27 (1.35)	4.77 (2.69)	3.99 (3.87)	3.34 (0.70)	4.16 (4.19)	4.23 (2.57)	3.11 (0.68)	4.36 (3.29)
	1 year	3.53 (2.24)	4.42 (2.06)	3.10 (0.38)	3.48 (1.85)	3.05 (0.39)	4.16 (1.17)	2.86 (0.46)	4.02 (2.07)
	3 years	3.11 (1.00)	4.33(2.85)	3.10 (0.46)	3.13 (1.12)	3.02 (0.54)	3.91 (2.30)	2.99 (0.51)	3.67 (2.00)
MP_ME_5.6: (%)	3 months	26.05 (20.97)	38.38 (28.32)	25.23 (25.54)	25.12 (19.97)	23.63 (17.31)	34.33 (27.01)	17.03 (19.43)	34.17 (22.59)
	1 year	23.39 (19.54)	34.82 (25.22)	17.92 (18.33)	31.26 (18.08)	19.32 (19.02)	25.83 (24.85)	12.15 (14.47)	30.83 (22.05)
	3 years	15.81 (21.07)	34.66 (24.75)	15.63 (10.48)	23.85 (24.66)	15.72 (11.45)	23.00 (23.31)	14.32 (11.70)	24.26 (23.31)
MP_ME_2.8: (%)	3 months	22.81 (8.54)	17.28 (12.97)	21.40 (9.49)	21.45 (10.16)	21.54 (9.11)	19.34 (11.34)	25.63 (8.74)	18.23 (10.18)
	1 year	22.19 (10.70)	16.81 (12.96)	26.17 (7.97)	17.57 (9.26)	25.45 (7.82)	21.61 (13.21)	26.64 (7.20)	19.73 (11.90)
	3 years	24.89 (9.19)	17.23 (10.06)	24.32 (5.51)	22.49 (10.32)	24.13 (5.84)	21.50 (9.83)	25.02 (7.18)	21.35 (9.40)

Statistically significant differences were not found.

capabilities. Furthermore, Classes II and III showed differences in relation to Class I for various ST parameters, including X50, ME_5.6, and cycle time. Class II individuals showed slightly worse average particle crushing capacity in the 3rd year, however, the ability to triturate coarse particles, and the time and number of cycles simultaneously showed improvements. The slightly worse crushing ability of Classes II and III may be related to the reduced time taken for chewing, as this can directly interfere with particle crushing (25, 26). In this context, the study by Van der Bilt (27) showed that subjects with good masticatory function, do not necessarily swallow food after a smaller number of cycles, as the ST is directly influenced by the physiology of the individual aside from the social context wherein the individual is included, as the social context can induce the patient to chew more quickly. In addition, the mandibular protrusion in Class III and maxillary protrusion in Class II individuals may also be responsible for these differences in MF in the 3rd year of IMO function.

It is well-known that sex can influence MF even after rehabilitation with IMO. In our study population, males still showed changes in particle homogenization capacity after 3 years. Hatch et al. (28) found that sex was the factor that most influenced the bite force, mainly due to the larger thickness of the masseter which is the main contributing factor to a greater bite force. Thus, the improvement in the particle homogenization for male individuals may be related to development of the masseter during medium-term IMO use. In this context, the literature showed that the bite force and the MF continue to improve over 3 years of IMO use (29), and show that after treatment with implants there is a long-term neuromuscular adaptation, and report an increase in myodynamic parameters and electromyography, approximating the values of dentate individuals (30), corroborating our results.

Presently, little is known about the influence of facial morphology on OHRQoL and patient satisfaction. Faot et al. (10) observed that treatment with IMO positively impacts OHRQoL after 3 months of rehabilitation, especially in the Oral Comfort domain. In the present study, however it was observed that FP can still influence OHRQoL medium-term, since individuals with Dolichofacial and Brachyfacial features reported distinct scores in various domains in the 3rd year, where Dolichofacial individuals reported a worse score (11%) in the Oral Comfort domain and Brachyfacial individuals reported a worse score (8%) in the Appearance domain compared to the reference group (Mesofacial individuals). In terms of ASD, only Class III still shows significantly lower scores (7%) than Class I individuals in the Oral Comfort domain after 3 years. While Faot et al. (10) found no differences in subjective perception in both types of PF and ASD 3 months after loading the IMO our results indicate that on the medium- to long-term, OHRQoL is influenced by different facial patterns and anteroposterior discrepancies. Moreover, age influenced the OHRQoL regardless of facial patterns, mainly in the Pain, Oral Comfort and General Performance domains. These results are in accordance with Schuster et al. (31) wherein the authors observed that individuals aged ≥65 years reported worse domain scores than individuals aged <65 years, reflecting a decrease in OHRQoL with increasing age. The effect size analysis reveals that Mesofacial and Class III individuals reported

TABLE 5 | Swallowing threshold outcomes (mean ± standard deviation) over time according to facial pattern, anteroposterior skeletal discrepancy, and sex (Wilcoxon-paired test).

		Facial pattern			Anteroposterior skeletal discrepancy			Sex	
		Mesofacial	**Brachyfacial**	**Dolichofacial**	**Class I**	**Class II**	**Class III**	**Male**	**Female**
ST_X50: (mm)	3 months	3.81 (0.98)	4.53 (0.98)	3.65 (0.89)	3.72 (1.15)	3.70 (0.98)	4.24 (0.99)	**3.32 (0.93)***	4.23 (0.97)
	1 year	3.47 (1.33)	4.21 (1.34)	3.49 (0.94)	3.91 (1.29)	3.33 (0.94)	3.85 (1.37)	**3.06 (0.72)***	3.99 (1.31)
	3 years	3.07 (1.06)	4.34 (1.35)	3.41 (0.60)	3.29 (1.23)	3.39 (0.66)	3.73 (1.29)	2.92 (0.67)	3.78 (1.17)
ST_B	3 months	2.88 (0.93)	4.93 (4.83)	3.12 (1.21)	3.17 (1.78)	3.19 (1.33)	4.05 (4.00)	3.05 (1.74)	3.81 (3.31)
	1 year	3.43 (1.92)#	3.68 (2.21)	2.93 (0.61)	3.70 (2.36)#	2.97 (0.52)	3.41 (1.87)	2.87 (0.60)#	3.55 (1.98)
	3 years	2.64 (0.80)#	3.40 (1.99)	2.83 (0.37)	2.70 (0.89)#	2.79 (0.44)	3.11 (1.58)	2.52 (0.44)#	3.10 (1.34)
ST_ME_5.6: (%)	3 months	18.11 (14.64)	32.34 (19.29)	13.69 (14.04)	17.58 (16.58)	15.40 (15.01)#	26.61 (18.63)	11.66 (12.25)#	25.25 (18.04)
	1 year	14.88 (24.05)	31.01 (24.85)	12.14 (17.06)	26.87 (29.00)	9.72 (15.88)#	21.10 (23.28)	7.15 (11.16)#	24.16 (25.28)
	3 years	9.66 (15.06)	26.07 (21.85)	13.75 (15.51)	18.50 (21.67)	9.62 (9.35)	18.04 (20.31)	6.49 (4.90)	20.00 (20.48)
ST_ME_2.8: (%)	3 months	22.88 (7.88)	18.26 (11.39)	24.30 (7.98)	21.94 (8.61)	25.75 (8.61)	19.50 (9.65)	25.76 (8.15)	20.21 (9.35)
	1 year	22.85 (11.53)	20.63 (11.01)#	27.50 (10.95)	24.37 (14.65)	27.76 (9.28)	20.69 (10.24)	29.27 (9.44)	21.25 (11.26)
	3 years	25.00 (9.59)	16.33 (10.49)#	28.54 (7.46)	21.35 (9.86)	28.94 (6.62)	21.83 (11.55)	26.12 (5.78)	22.65 (11.66)
Time: (sec)	3 months	59.48 (31.03)	56.14 (23.59)	62.98 (19.09)#	57.05 (23.28)	62.04 (15.64)	60.83 (30.08)	62.40 (25.07)	59.19 (24.58)
	1 year	60.40 (36.86)	56.95 (22.96)	56.18 (13.24)	47.32 (12.79)	53.82 (12.71)	65.98 (34.50)	55.05 (13.70)	59.22 (30.06)
	3 years	56.37 (14.02)	60.37 (26.13)	47.46 (19.25)#	57.53 (24.67)	49.05 (20.49)	56.11 (17.31)	63.97 (18.13)	50.10 (19.59)
Cycles	3 months	69.73 (37.73)	60.31 (23.61)	73.71 (29.36)	70.64 (35.62)	73.33 (28.61)#	63.42 (30.35)	79.46 (36.74)	63.07 (27.04)
	1 year	65.71 (37.09)	62.25 (16.28)	56.62 (15.31)	51.78 (14.96)	57.67 (15.50)#	69.17 (32.23)	61.83 (14.66)	61.52 (28.96)
	3 years	63.31 (25.93)	67.40 (28.10)	57.08 (18.18)	63.89 (31.82)	60.00 (18.38)	62.94 (23.36)	74.18 (24.31)	56.92 (22.12)

#*Shows statistically significant difference according to Wilcoxon-paired (p = 0.05).*

**Shows statistically significant difference according to Wilcoxon-paired test using Bonferroni correction of the P-values (p = 0.0166).*

Exact p values found according to the intragroup comparisons: Mesofacial= ST_B (1–3 y, p = 0.023); Brachyfacial = ST_ME_2.8 (1–3 y, p = 0.022); Dolichofacial = Time (3 m−3 y, p = 0.050); Class I = ST_B (1–3 y, p = 0.036); Class II = ST_ME_5.6 (3 m−1 y, p = 0.034), Cycles (3 m−1 y, p = 0.041); ***Male = ST_X50 (3 m–1 y, p = 0.015)****, ST_B (1–3 y, p = 0.028), ST_ME_5.6 (3 m−1 y, p = 0.019).*

a reduction in the General Performance domain in the 3rd year compared to the first. However, in general facial patterns had limited influence on the OHRQoL outcomes, as most domains maintained an average score > 0.7, reflecting overall satisfaction with the treatment.

The limitations of this study include the lack myographs, especially of the masseter, and bite force measurements, as these directly influence MF. Another limitation relates to the lack of studies available for direct comparison of these results, due to the scarcity of studies in literature assessing medium-term effects of MF and OHRQoL as a function of facial morphology (FP and ASD). The present study assessed a relatively small number of patients (n = 40) given the amount of parameters needed to describe these relationships. Extrapolation of our results to different populations should be done with caution. More studies with larger sample sizes, more diverse sample populations, and assessment of more parameters related to mastication are required to understand the medium-term relationships between oral health-related quality of life, mastication, and facial morphology.

CONCLUSION

The masticatory performance and oral health-related quality of life parameters of implant mandibular overdenture users change over time as a function of facial pattern, anteroposterior skeletal discrepancies, and sex. In our study population, the differences in facial morphology continued to influence the masticatory function and oral health-related quality of life in the 3rd year of implant mandibular overdenture function, and age can influence some OHRQoL domains; brachyfacial individuals benefited least from rehabilitation with IMO, as several masticatory outcomes deteriorated, such as particle homogenization and masticatory efficiency (ME_2.8).

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article and further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Research Ethics Committee of the Faculty of Dentistry UFPel, protocol (No. 69/2013). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FF, AP, and AM: conceptualization and project administration. AP, AS, RM-M, AM, LP, and OC-J: methodology, patient's treatment, and clinical follow-ups. AP, AS, RM-M, and AM: writing—original draft preparation, software, data curation, and visualization. FF, LP, OC-J, and AD: investigation. FF, LP, and OC-J: formal analysis, resources, and supervision. FF, AP, AS, RM-M, and AD: writing—review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil—Finance Code 001.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 02 March 2021
doi: 10.3389/fnut.2021.623507

Higher Masticatory Performance and Higher Number of Chewing Strokes Increase Retronasal Aroma

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Mastication is a physiological process whereby food is comminuted and mixed with saliva to form a swallowable bolus; it is also the initial process for retronasal aroma that is released from foods to receptors in the nose. However, the influence of mastication state on retronasal aroma is poorly understood. The purpose of this study was to investigate the relationship between aroma concentration and factors related to mastication state. The study design was an analytical observational study. Twelve male volunteers (age, 26.5 ± 2.7 years) were recruited and divided into five and seven participants in the low and high masticatory performance groups, respectively. The stimulated salivary flow rate was measured while participants chewed paraffin wax. First, an odor sensor was placed in the nostril, and the aroma concentration was measured over time as participants chewed an orange-flavored gummy jelly standardized for masticatory performance assessment until swallowing; chewing strokes were counted to determine swallowing thresholds. Next, participants were instructed to chew the gummy jelly for a certain number of strokes (i.e., 50 or 100% of swallowing thresholds, as well as 30 strokes) and expectorate the jelly without swallowing. The surface area of comminuted jelly at 30 chewing strokes was defined as masticatory performance. Maximum and slope of aroma concentration, surface area, number of chewing strokes, and stimulated salivary flow rate were compared between low and high masticatory performance groups. Statistical significance was set at $\alpha = 0.05$. At 30 chewing strokes, the maximum aroma concentration and the slope were significantly greater in the high masticatory performance group than in the low masticatory performance group. There was a positive correlation between the maximum aroma concentration and the number of chewing strokes with aroma release in both groups. No significant correlation was found between the maximum aroma concentration and the stimulated salivary flow rate. However, multiple regression analysis (with aroma concentration as a dependent variable) showed that the increase in surface area, the number of chewing strokes, and the stimulated salivary flow rate were significant explanatory variables. The results suggested that retronasal aroma was influenced by mastication state and salivary flow rate during chewing.

Keywords: retronasal aroma, masticatory performance, chewing strokes, swallowing threshold, stimulated salivary flow

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Eating Behavior, a section of the journal Frontiers in Nutrition

Received: *30 October 2020*
Accepted: *28 January 2021*
Published: *02 March 2021*

Citation:
Okawa J, Hori K, Yoshimoto T, Salazar SE and Ono T (2021) Higher Masticatory Performance and Higher Number of Chewing Strokes Increase Retronasal Aroma. Front. Nutr. 8:623507. doi: 10.3389/fnut.2021.623507

INTRODUCTION

Mastication is a physiological process whereby food is comminuted, mixed with saliva, and formed into a bolus that can be swallowed safely (1). Mastication also serves to release aromas from foods, which are transported through the pharynx to the nose and perceived (these comprise retronasal aromas). Masticatory function and retronasal perception during food intake have been studied in relation to eating behavior that leads to metabolic syndrome and obesity (2–6). However, the influence of an individual's physiological mastication state (i.e., mastication condition) on retronasal aroma is poorly understood.

The aromas from foods comprise volatile compounds that are released from the food surface by diffusion between food and air phases (7–9). The surface area between these phases is formed as a result of food comminution by chewing (10) and is increased by a large number of chewing strokes (11, 12). Masticatory performance is an objective parameter for evaluating the state of food comminution by chewing; high masticatory performance enables comminution of foods more efficiently with a small number of chewing strokes (12). Tarrega et al. investigated the aroma released from cheeses with the chewing activity (13) and the masticatory performance measured using a silicone rubber (14); however, the aroma release was related to the chewing behavior, but relationship with masticatory performance was not found. In recent years, masticatory performance has been assessed by measuring the increase in surface area of standardized and flavored gummy jelly after 30 chewing strokes (15–17); this parameter is reportedly associated with metabolic syndrome (4).

Furthermore, the properties of food boluses are influenced by the mixing with saliva (18, 19). Saliva is secreted by stimulation during chewing, which facilitates food bolus formation (20). A reduction in stimulated salivary flow rate reduces masticatory performance (21). In gelatin-based foods such as the gummy jelly, the reduction in food hardness caused by saliva leads to increased aroma release (8). However, the aroma dissolves in saliva and is thus attenuated (22). Feron et al. (23) reported that the stimulated salivary flow rate is negatively correlated with aroma release during mastication.

During the progression of mastication, a swallowable food bolus is formed until the swallowing reflex is triggered; this constitutes the swallowing threshold (12, 24). The masticatory performance and stimulated salivary flow rate also influence the number of chewing strokes until the swallowing threshold is reached (25).

Therefore, physiological parameters such as masticatory performance, number of chewing strokes, and salivary flow rate interact with each other and may be associated with retronasal aroma. We constructed the following hypothesis regarding the physiological parameters that might influence retronasal aroma release while chewing the gummy jelly: (1) higher masticatory performance and more chewing strokes lead to increased retronasal aroma and (2) high stimulated salivary flow rate reduces retronasal aroma. To test this hypothesis, we measured the aroma concentration (through the nostril) over time while chewing the gummy jelly in participants with different levels of masticatory performance. This study was performed to clarify differences in retronasal aroma release depending on masticatory performance and to investigate the relationships of aroma concentration with masticatory performance, number of chewing strokes, and stimulated salivary flow rate.

MATERIALS AND METHODS

Participants

The participants in this study were 12 male volunteers (age, 26.5 ± 2.7 years; body mass index, min–max = 17.8–30.9) who had complete dentition; none had a history of dysphagia, neuromuscular disorders, chronic sinus infection (nasal obstruction), respiratory diseases, temporomandibular disease, taste and smell disorder, or dry mouth. This study conformed to the standards of the Declaration of Helsinki and was approved by the Ethics Committee of Niigata University Faculty of Dentistry (23-R35-03-01). All volunteers provided written informed consent to participate in this study.

Test Samples

A gummy jelly (5.5 g, 20 × 20 × 10 mm, UHA Mikakuto Co., Ltd., Osaka, Japan), which had been standardized for masticatory performance assessment, was used as a test food (15–17). The main component of the jelly was gelatin; moreover, the jelly contained orange flavor and the β-carotene pigment. Paraffin wax without any taste or odor (1.0 g, OralCare. Inc, Tokyo, Japan) was used for measurement of stimulated salivary flow rate.

Equipment

Odor Sensor

During chewing, aroma concentration was measured over time using a portable odor sensor (XP-329IIIR; New Cosmos Electric Co., Ltd., Tokyo, Japan). The odor sensor absorbed at a suction flow rate of 450 ± 150 ml/min and output aroma concentration over time; it was equipped with indium oxide-based high-sensitivity hot-wire semiconductor sensors. When the absorbed odor compounds adhered to the sensors, the resistance level was reduced. This change in resistance level was used as the output voltage deviation of the bridge; it was converted to a numerical representation of aroma concentration over time. Because the sensor specificity was not limited to particular aromatic compounds, multicompound odors could be detected. The output aroma concentration was recorded as a relative value in relation to a calibration odor without any units. The calibration odor was generated when the aromatic compound gas adhered to and was removed by a built-in active carbon filter in the device. The sampling frequency was 2 Hz. The data output from the odor sensor were recorded by the computer in real time using MATLAB version R2016b (The MathWorks Inc., Natick, MA, USA).

Masticatory Performance Measurement

Masticatory performance was evaluated based on the increase in surface area of the comminuted gummy jelly using a smartphone (SH-M05, Android version 8.0.0, Sharp Corporation, Osaka, Japan) (17). This is a programming designed based on the

previous method (15, 16) for evaluating the increase in surface area from the elution of β-carotene. The comminuted gummy jelly was washed with water and then transferred to a dedicated box containing 30 ml of water. The increase in surface area of the comminuted gummy jelly was calculated using a photograph taken by a smartphone camera without flash, following image processing (17).

Electromyography

The muscle activities of masseter and suprahyoid muscles during chewing were recorded using a wireless surface electromyography system (Trigno Wireless EMG System; Delsys Inc., Natick, MA, USA), which uses an active electrode with a fixed inter-electrode distance of 10 mm. The electrodes were placed in the left and right masseter muscles and the suprahyoid muscle group; the positions were determined by muscle palpation. The sampling frequency was 1 kHz.

Throat Microphone

The swallowing sound was measured by a throat microphone (SH-12jk, NANZU ELECTRIC Co., Ltd., Sizuoka, Japan), which was placed in the neck region. The sampling frequency was 40 kHz.

Synchronizing System

Electromyography activity and sound were recorded with an analog-to-digital converter (Power Lab, AD Instruments, New South Wales, Australia). A synchronizing signal was recorded in both PowerLab and MATLAB, then used to synchronize all data. The stored data in PowerLab were imported to MATLAB, and the time axes were matched based on the synchronizing signal.

Data Collection

The test for stimulated salivary flow rate was carried out once before measurement of aroma concentration to prevent stimulation from chewing the gummy jelly. Before saliva collection, each participant chewed the paraffin wax until it became soft, then swallowed the saliva in the oral cavity. Beginning at that point, the participant chewed the paraffin wax continuously for 5 min. The saliva secreted during the measurement was expectorated into a measuring cup at short intervals (26).

Next, the participant was asked to sit in a chair. An odor sensor with a 15-cm nasal tube (Nasal Cannula Ref#302-E; 2-mm inner diameter; Unomedical, Inc., McAllen, TX, USA) attached to the nose piece was placed in the participant's both nostrils (27).

In the present study, the following two experiments were conducted to measure aroma concentration during chewing:

Experiment (1) Aroma concentration during free intake. Each participant was instructed to chew and swallow the gummy jelly freely. The aroma concentration was measured from the start of chewing the gummy jelly until the first swallowing. The number of chewing strokes before swallowing was recorded as the swallowing threshold.

Experiment (2) Aroma concentration and increase in surface area for a certain number of chewing strokes. The numbers of chewing strokes were set to 50 and 100% of swallowing thresholds, as well as 30 strokes. The aroma concentration was measured from the start of chewing the gummy jelly until the designated number of chewing strokes was reached. Then, the comminuted gummy jelly was expectorated without swallowing, and the increase in surface area was calculated by image analysis.

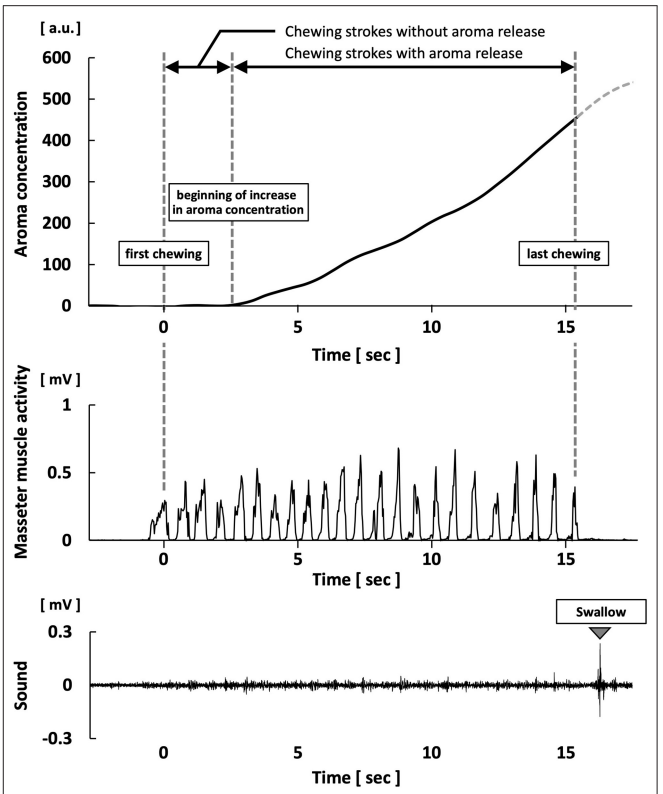

FIGURE 1 | Waveforms of aroma concentration, masseter muscle activity, and swallowing sound during gummy jelly intake.

To calibrate aroma concentration, each participant was instructed to place the gummy jelly in the oral cavity 15 s before the start of each measurement and to breathe through the nose without chewing. A previous investigation confirmed that no aroma was released in this situation (27). The average aroma concentration during calibration was set as the baseline. After each measurement, participants were asked to rinse their mouths repeatedly with 5 ml of water and to swallow the water to clear the pharynx, then to wait until the aroma concentration decreased to baseline levels. Each of these four tasks (experiments 1 and 2) was performed three times, for total of 12 trials. In experiment 2, the order of tasks was chosen at random.

Data Analysis

Increase in Surface Area of Comminuted Gummy Jelly and Masticatory Performance

For each participant, the average increase in gummy jelly surface area was calculated in each task; the masticatory performance was defined as the average value after chewing 30 strokes. The masticatory performance of each participant was evaluated with reference to the methods used by Kosaka et al. (28) and Nokubi

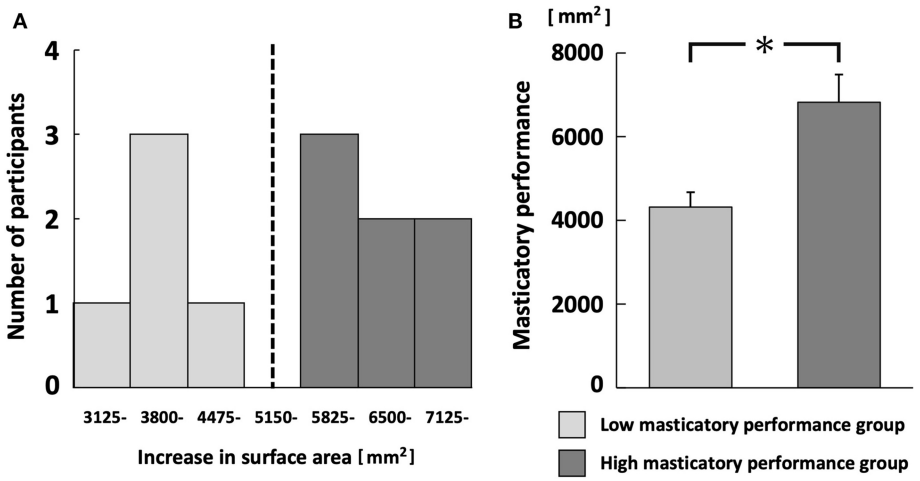

FIGURE 2 | Histogram of masticatory performance **(A)** and comparison between low and high masticatory performance groups **(B)**. Black dotted line in **(A)** separates low and high masticatory performance groups. *$p < 0.05$.

et al. (29). Participants were divided into two groups: high masticatory performance (high MP group) and low masticatory performance (low MP group), using a cutoff value of 5,825 mm^2 from previous studies (28, 29).

Stimulated Salivary Flow Rate

For each participant, the stimulated salivary flow rate was calculated as the volume of saliva expectorated during chewing, in milliliters per minute.

Aroma Concentration

In preparation for the analysis, the measured aroma concentration was subtracted from the baseline level in the raw data; this was used as the calibrated aroma concentration. The maximum aroma concentration during chewing was calculated from the aroma concentration after calibration. The average slope was calculated by dividing the maximum aroma concentration by the duration of chewing strokes with aroma release; this was measured in aroma concentration *per second* (**Figure 1**).

Chewing Strokes

The electromyography waveform of the masseter muscle was converted to a full-wave rectified waveform; the root mean square (with a window length of 100 ms) was calculated for smoothing. The local maximum of the waveform was determined by software analysis as the masseter muscle burst due to chewing. The number and position of bursts during chewing were recorded as the number of chewing strokes and the timing of chewing, respectively (30). The swallowing was identified from the sound and the electromyographic waveform of the suprahyoid muscles. The swallowing threshold was defined as the number of chewing strokes during the period from the start of chewing (before the first swallow) and was averaged three times for each participant. During free intake, a calibration period of 15 s was recorded; then, the number of chewing strokes without aroma release was counted from the start of chewing to the onset of increasing aroma concentration.

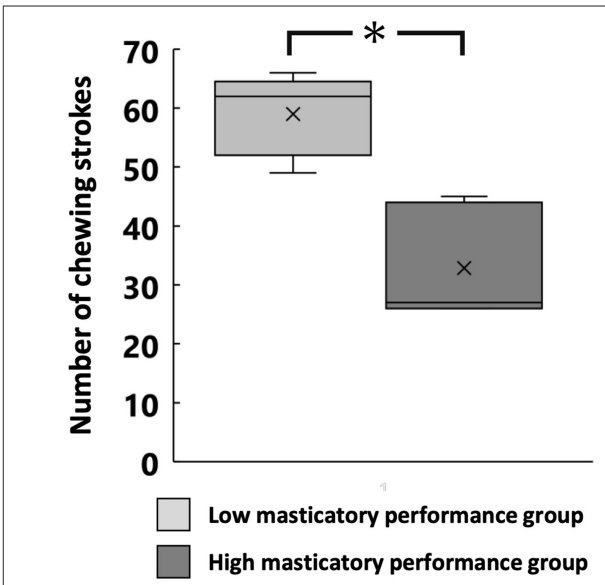

FIGURE 3 | Number of chewing strokes at swallowing threshold for each group. *$p < 0.05$.

Statistical Analysis

The sample size was calculated from preliminary experiment data with $\alpha = 0.05$ and detection power = 0.8. The numbers of participants in the low and the high MP group were 2 and 3. The variance normality and equality were examined in the low and high MP groups for each of the following parameters: increase in surface area of gummy jelly, the number of chewing strokes, and the aroma concentration. When variance normality and equality

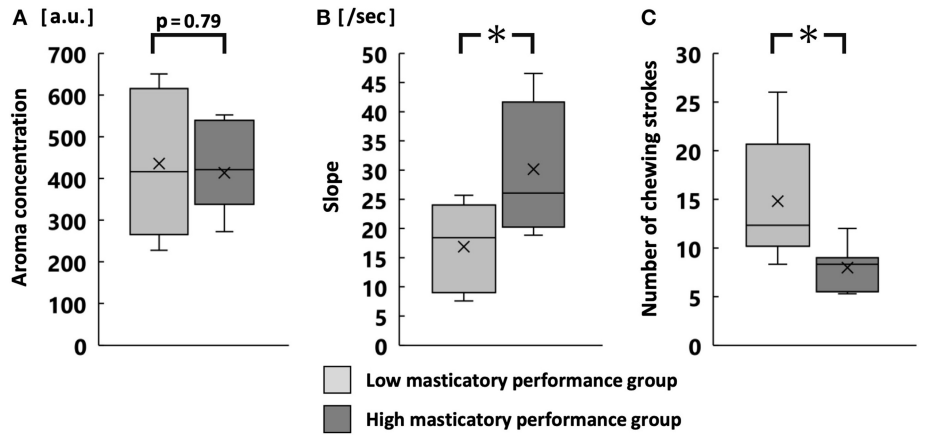

FIGURE 4 | Maximum aroma concentration **(A)**, slope **(B)**, and number of chewing strokes without aroma release **(C)** during free intake. *$p < 0.05$.

were present, Student's *t*-test was used for further analysis. When variance normality and equality were absent, the Mann–Whitney *U*-test was used for further analysis. Correlations were calculated using either the Pearson correlation coefficient or the Spearman rank correlation coefficient. The correlation between the number of chewing strokes and the aroma concentration, which were data for each measurement in Experiment 2, was calculated within-participant and within-group. The correlation between the increase in surface area of gummy jelly and the aroma concentration, which were averaged three times for each participant in Experiment 2 was were also calculated. Furthermore, multiple regression analysis with the forward-backward stepwise selection method was performed; aroma concentration was the dependent variable, while masticatory performance, number of chewing strokes, and stimulated salivary flow rate were independent variables. Statistical significance was set at $\alpha = 0.05$. All statistical analyses were performed with IBM SPSS Statistics, version 23.0j (IBM Japan, Ltd., Tokyo, Japan).

RESULTS

Masticatory Performance

There were five and seven participants in the low and high MP groups, respectively. The mean masticatory performances were 6,823 ± 715 mm^2 (range, 5,927–7,748 mm^2), 4,319 ± 396 mm^2 (range, 3,757–4,858 mm^2), respectively ($p < 0.001$; **Figures 2A,B**). There was a weak correlation between masticatory performance and stimulated salivary flow rate, but this was not statistically significant ($r = 0.40$, $p = 0.20$).

Swallowing Threshold

The swallowing threshold was significantly lower in the high MP group than in the low MP group (32.7 ± 8.2 and 59.0 ± 6.2, respectively, $p < 0.05$; **Figure 3**).

Aroma Concentration During Free Intake

There was no significant difference in the maximum aroma concentration during free intake (**Figure 4A**). However, the average slope was significantly greater in the high MP group than in the low MP group ($p < 0.05$; **Figure 4B**). The number of chewing strokes without aroma release was significantly smaller in the high MP group than in the low MP group ($p < 0.05$; **Figure 4C**). **Supplementary Figure 1** shows averaged time-release plots of the aroma concentration for both groups.

Aroma Concentration for a Specific Number of Chewing Strokes

At 30 chewing strokes, the maximum aroma concentration and average slope were significantly greater in the high MP group than in the low MP group ($p < 0.05$; **Figures 5A,B**). At chewing strokes equal to 100% of the swallowing threshold, there was no significant difference in the maximum aroma concentration and the average slope (**Figures 5C,D**).

The increase in surface area of comminuted gummy jelly at chewing strokes equal to 100% of the swallowing threshold was significantly greater in the high MP group than in the low MP group ($p < 0.05$; **Figure 6**). There was no significant difference in the maximum aroma concentration between chewing strokes equal to 100% of the swallowing threshold and free intake (**Figure 7**).

Correlations of Aroma Concentration With Chewing Strokes, Surface Area, and Stimulated Salivary Flow Rate

There was a positive correlation between the maximum aroma concentration and the number of chewing strokes with aroma release in both the low MP group ($r = 0.69$, $p < 0.01$; **Figure 8A**) and high MP group ($r = 0.58$, $p < 0.01$; **Figure 8B**). This positive correlation was also observed in each participant; the average value of the correlation coefficient in each participant was 0.80 ± 0.13. Furthermore, the slope of linear regression was significantly greater in the high MP group (19.6 ± 9.9) than in the low MP group (7.2 ± 1.9; $p < 0.05$).

A significant positive correlation was observed between the maximum aroma concentration and the increase in surface

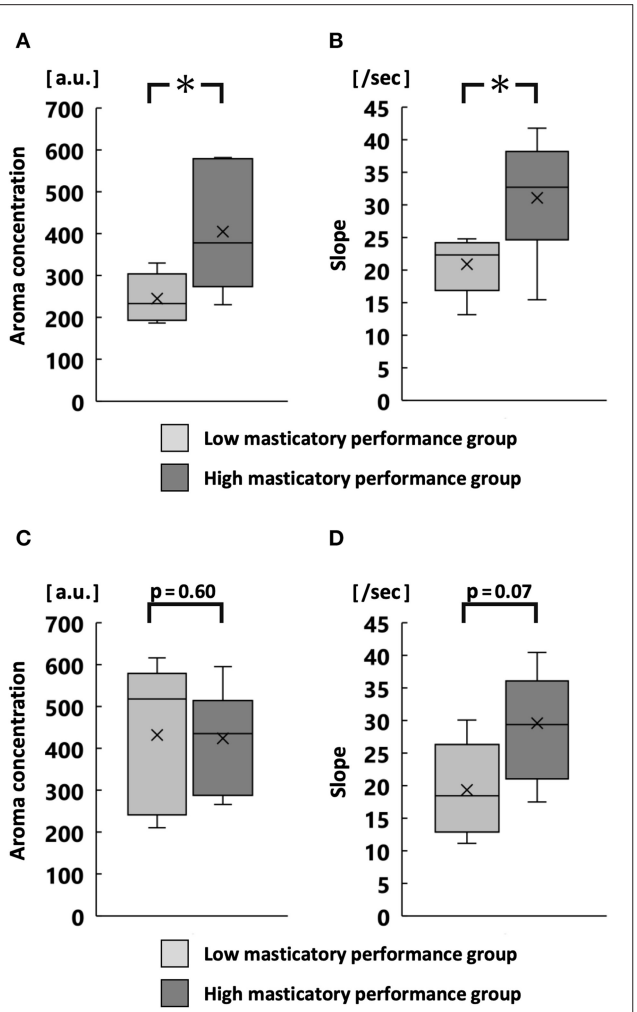

FIGURE 5 | Maximum aroma concentration and slope at 30 strokes **(A,B)** and at chewing strokes equal to 100% of swallowing threshold **(C,D)**. *$p < 0.05$.

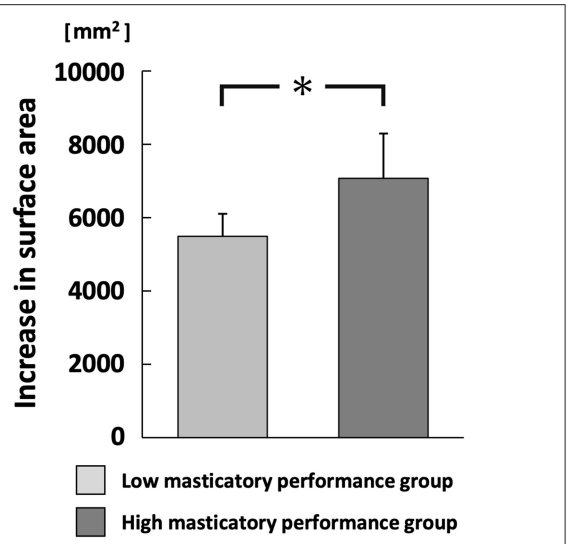

FIGURE 6 | Increase in the surface area of comminuted gummy jelly at chewing strokes equal to 100% of swallowing threshold. *$p < 0.05$.

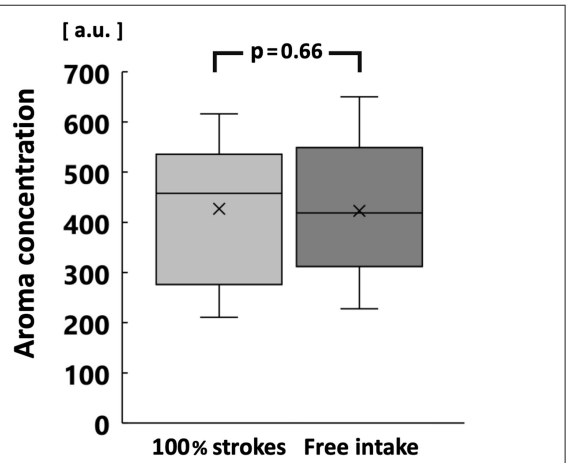

FIGURE 7 | Comparison of maximum aroma concentration between chewing strokes equal to 100% of swallowing threshold and free intake. No significant difference was observed.

area ($r = 0.55$; $p < 0.01$; **Figure 9**). No significant correlation was found between the maximum aroma concentration and stimulated salivary flow rate.

Multiple Regression Analysis

Table 1 shows the results of multiple regression analysis; aroma concentration was the dependent variable, while masticatory performance, number of chewing strokes, and stimulated salivary flow rate were independent variables. Masticatory performance, number of chewing strokes, and stimulated salivary flow rate were all identified as significant factors ($R^2 = 0.44$).

DISCUSSION

The present study investigated the dynamics of retronasal aroma during the oral processing of gummy jelly in various physiological mastication states. The results showed that the aroma concentration (measured through the nostril) differed depending on masticatory performance and the number of chewing strokes. Furthermore, multiple regression analysis suggested that aroma concentration during food intake may be related to the increase in surface area of comminuted jelly, the number of chewing strokes, and the stimulated salivary flow rate.

Methodological Considerations

The aroma concentration and masseter muscle activity were measured simultaneously during gummy jelly chewing, while masticatory performance was measured by examination of chewed gummy jelly. These methodologies allowed investigation of the relationship between retronasal aroma and physiological mastication state. Previous studies had some limitations in measuring masticatory function and aroma

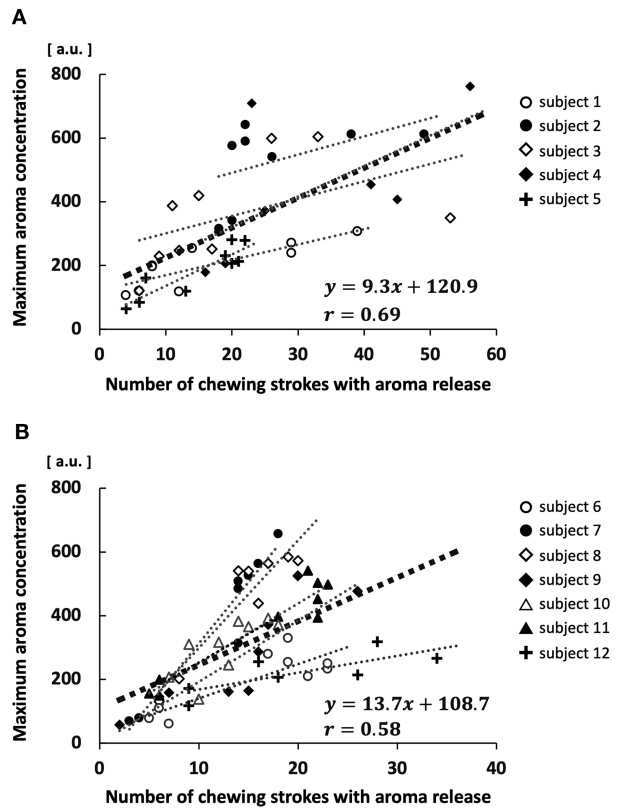

FIGURE 8 | Correlation between maximum aroma concentration and number of chewing strokes with aroma release in each participant. **(A)** High masticatory performance group. **(B)** Low masticatory performance group.

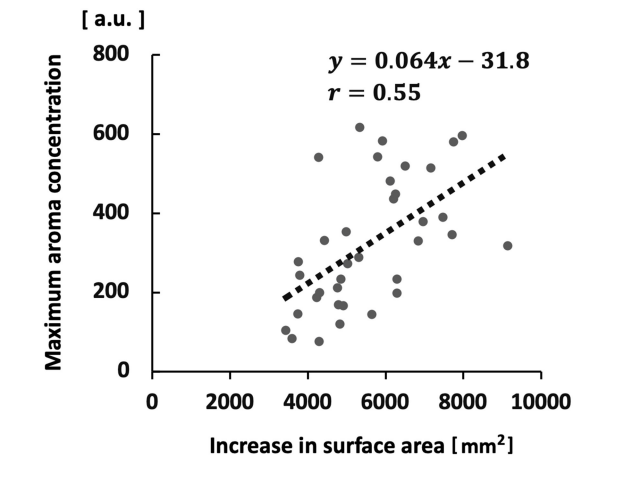

FIGURE 9 | Correlation between maximum aroma concentration and increase in surface area.

release measurements: masticatory function was measured by indirect evaluation such as electromyography and occlusal force tests, or aroma and masticatory function were measured by different tasks. In this study, masticatory performance was used to evaluate masticatory function, which is a quantitative and direct evaluation of measuring the comminuted sample and is a common technique in the dental field. Electromyography and occlusal force tests are parameters for estimating masticatory function, however they are indirect evaluation for investigating the state of muscles. Therefore, the masticatory performance using gummy jelly was adopted, which can directly and simultaneously evaluate the masticatory function that occurs as an operation of the state of muscles.

Chewing strokes equal to 100% of the swallowing threshold was used because mastication that exceeds the swallowing threshold requires swallowing suppression. The swallowing threshold varies greatly among individuals (12, 25); the present study also showed different swallowing thresholds depending on masticatory performance. Therefore, by setting the chewing strokes below the swallowing threshold for each participant, measurement during physiological mastication could be ensured; 30 strokes is an established number for assessment of masticatory performance using the gummy jelly (4, 21). The average swallowing threshold for the high MP group in this study was 32.7, which was not considerably different from 30.

Masticatory Performance and Swallowing Threshold

The number of chewing strokes at swallowing threshold was lower in the high MP group than in the low MP group. In both groups, the food was crushed by efficient chewing and quickly formed into a swallowable bolus. However, the low MP group showed a small increase in the surface area of gummy jelly expectorated after chewing strokes equal to 100% of the swallowing threshold, although the number of chewing strokes was greater than in the high MP group. Participants with low masticatory performance increase the number of chewing stroke at the swallowing threshold, however they are not always able to comminuted the bolus into small pieces. These results were similar to the findings reported by Fontijn-Tekamp et al. (12).

Relationships of Aroma Concentration With Masticatory Performance and Chewing Strokes

The slope of aroma concentration was significantly greater in the high MP group than in the low MP group. In model mouth systems, fast crushing foods are increase aroma release (31, 32). In this study, because the high MP group had a high food crushing efficiency, the area of aroma release rapidly increases, which increases the slope size. Therefore, the maximum aroma concentration was higher in the high MP group than in the low MP group after 30 strokes.

In contrast, there was no significant difference in maximum aroma concentration between groups during free intake or when using chewing strokes equal to 100% of the swallowing threshold. The low MP group had a higher number of chewing strokes equal to 100% of the swallowing threshold, compared with the high MP group. Large number of chewing stroke and long chewing duration are reportedly increase aroma intensity (13, 33). However, the increase in surface area of the gummy jelly at

TABLE 1 | Multiple regression analysis of aroma concentration.

Independent variables	B	β	SE	*p*	95% CI	
(Intercept)	2.96		52.25	0.96	−100.70	106.61
Increase in surface area	0.04	0.36	0.01	<0.001	0.02	0.06
Number of chewing strokes	6.36	0.46	1.11	<0.001	4.15	8.56
Stimulated salivary flow rate	−40.74	−0.31	10.71	<0.001	−61.98	−19.49

$R^2 = 0.44$.
CI, confidence interval; SE, standard error.
Dependent variable is aroma concentration.

chewing strokes equal to 100% of the swallowing threshold was significantly smaller in the low MP group than in the high MP group. Therefore, for both reasons, there was no difference in maximum aroma concentration between groups. These findings imply that both the increase in surface area and the number of chewing strokes influence the aroma concentration. Our multiple regression analysis supported these findings.

Chewing Strokes Without Aroma Release

Aroma concentration was not detected at the start of chewing the gummy jelly. During free intake, the number of chewing strokes in this period was lower in the high MP group than in the low MP group. Participants were required to expend considerable effort to chew the gummy jelly because of its high hardness. Therefore, the gummy jelly was not comminuted sufficiently during early mastication; sufficient surface area for aroma release could not be generated. In addition, respiratory rhythm is reportedly perturbed in the early stage of chewing solid food (34), while an increase in airway resistance has been observed due to narrowing of the velo-pharynx isthmus in soft palate elevation during chewing (35). The high MP group might have been able to accomplish the initial gummy jelly crushing with high-effort chewing by using a smaller number of chewing strokes, thus initiating early aroma release.

Relationship Between Aroma Concentration and Stimulated Salivary Flow Rate

Masticatory performance tended to increase as the stimulated salivary flow rate increased, but there was no significant correlation between these parameters. There was also no significant correlation between the maximum aroma concentration and the stimulated salivary flow rate. Saliva changes the physical properties of gelatin, which is the main content of the gummy jelly; this leads to increased aroma release (8). However, Odake et al. (22) reported that the aroma component dissolves in saliva, resulting in a reduced aroma concentration and diminished aroma release. Saliva has both positive (8) and negative (22, 23) influences on retronasal aroma; the stimulated salivary flow rate was presumed not to be substantially influenced by means of a simple correlation. However, multiple regression analysis showed the stimulated salivary flow rate was negatively correlated with aroma concentration. Saliva may require simultaneous application with chewing to affect the retronasal aroma. The effect of the dissolution of aroma in saliva may be stronger than the change in physical properties during chewing.

Limitations

The sample size of this study was a small and the gender was only male. Since the masticatory performance varies depending on gender (36), further investigation with other genders is necessary. In addition, no consideration was given to the body constitution. There are several researches investigating the relationship between retronasal and obesity and body mass index (BMI) (5, 6, 37, 38). Although their association is often unclear, BMI and retronasal aroma can be interrelated factors. Larger sample sizes and adjustments are needed to investigate factors of the genders and the body constitution such as BMI and body composition.

The measurement of stimulated salivary flow rate in this experiment was not performed using saliva secreted during gummy jelly intake. The gummy jelly dissolves in saliva during chewing, which causes difficulty in correct measurement of saliva volume. Therefore, the analysis of stimulated salivary flow rate was performed using paraffin wax (26). The standardized gummy jelly is useful for assessment of natural chewing and quantitative evaluation of masticatory performance, but further analyses are needed to clarify its usage in studies of saliva.

In experiment 2, the swallowing suppression facilitated measurement of the increase in surface area after expectoration of the gummy jelly. Notably, this also suppressed stage II transport that propels the food bolus into the oropharynx (39) and the soft palatal movement that causes oropharyngeal opening (35). In addition, homogeneity and particle size of food bolus were altered by suppression of stage II transport (40). In this study, the aroma concentration was similar between free intake (with swallowing) and 100% of the swallowing threshold (without swallowing). Although the swallowing suppression may not have a strong effect on retronasal, these changes might have altered the masticatory and respiratory patterns, thereby affecting aroma release and transport. Measurement of respiration while chewing the gummy jelly may have helped to avoid effects on aroma release and transport. However, such assessment would have required the connection of another sensor to the nostril, which might have interfered with aroma measurement. Therefore, this respiratory measurement was not performed.

Despite the above limitations, to the best of our knowledge, this is the only study to simultaneously measure retronasal

aroma and masticatory performance. Furthermore, the aroma concentration was correlated with the increase in surface area of gummy jelly, the number of chewing strokes, and the stimulated salivary flow rate. The findings of the present study suggested the presence of a new physiological state factor associated with retronasal aroma; this new physiological state factor is a combination of masticatory performance and number of chewing strokes. However, it should be noted that the aroma concentration was measured by the odor sensor and does not necessarily reflect the aroma perception. The relationships of masticatory function and retronasal aroma may also be associated with eating behaviors that cause obesity and metabolic syndrome; thus, further research is needed.

CONCLUSIONS

Food aroma concentration, measured from the nostril while chewing the gummy jelly, varied depending on masticatory performance. In addition, the increase in surface area of gummy jelly and the number of chewing strokes were positively correlated with aroma concentration, while the stimulated salivary flow rate was negatively correlated with aroma concentration. These physiological mastication states are associated with retronasal aroma during food intake.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of the Faculty of Dentistry of Niigata University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

This study was designed by JO and KH. The data were collected by JO and TY, and were analyzed by JO. The manuscript was drafted by JO and KH and was edited by TO. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by JSPS KAKENHI Grant Numbers 15K11194, 15KK0300, and 19K24115.

ACKNOWLEDGMENTS

We thank Ryan Chastain-Gross, Ph.D., from Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.623507/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 04 March 2021
doi: 10.3389/fnut.2021.642726

Development of an Iberian Chorizo Salted With a Combination of Mineral Salts (Seawater Substitute) and Better Nutritional Profile

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The present study evaluated the effect of salt reduction using a seawater substitutes, at the nutritional and mineral composition, its physicochemical, biochemical, microbiological, and sensory characteristics of Iberian chorizo, compared with one elaborated with low salt content (KCl) and another with a normal salt content (CTRL). To this end, three batches of chorizo were prepared [Treatment 1: CTRL, 100% NaCl; Treatment 2: KCl, 31% KCl, and Treatment 3: SC (Winbi®), <3% NaCl]. In KCl and SC chorizo lots, values of moisture, salt, and water activity (a_w) were significantly lower ($P < 0.05$) than in the CTRL chorizo. The chorizo with lower salt content presented higher proteolytic activity; with the nutritional declaration "reduced Na content "with Na values 25% lower than the CTRL. In addition, using this combination caused significant effects ($P < 0.05$) on the mineral composition of chorizo SC, allowing the inclusion of more nutritional and health claims in its labeling under legislation. The partial substitution of NaCl for KCl (31%), caused an increase in the gumminess, chewiness, and hardness of the chorizo. The SC chorizo lost the reddish hue typical of this sausage, although it was the best sensory valued by a panel of consumers. No differences were observed in the microbiological quality of the different batches of chorizo, always fulfilling the legally established microbiological criteria.

Keywords: sodium chloride replacement, nutrition properties, nutrition and health food labeling, sausage, mineral salt (Winbi)

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Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *16 December 2020*
Accepted: *08 February 2021*
Published: *04 March 2021*

Citation:
Tejada L, Buendía-Moreno L, Álvarez E, Palma A, Salazar E, Muñoz B and Abellán A (2021) Development of an Iberian Chorizo Salted With a Combination of Mineral Salts (Seawater Substitute) and Better Nutritional Profile. Front. Nutr. 8:642726. doi: 10.3389/fnut.2021.642726

INTRODUCTION

Salt has been a preservative in food for thousands of years. The discovery of other preservation methods, such as refrigeration, has allowed the reduction of this food additive, but it is still not enough (1). According to the World Health Organization, high blood pressure and cardiovascular diseases are the leading cause of death worldwide (2). One of the primary determinants involved in the origin of high blood pressure is the excessive consumption of sodium that is ingested in the diet as sodium chloride (common salt; NaCl), its intake linked to kidney diseases and increases in blood pressure (3). In Europe, the average daily consumption of common salt is estimated to be 8.11 g/day, well above the 5 g/day recommended (4).

Currently, the reformulation of food to improve its healthiness is an important aspect and a challenge for the food industry. Therefore, the framework of the European Green Pact and the strategy "from farm to fork," propose actions that, synergistically, help consumers in choosing healthy diets and sustainable. These measures comprise the establishment of nutritional profiles (with thresholds for nutrients such as fats, sugars, and salt) that regulate the use of nutritional and health claims contemplated in Regulation (EC) No. 1924/2006, as well as nutritional labeling on the front-of-package that makes it easier for consumers to understand the nutritional composition of foods.

Salt is a component widely used in the food industry, as it can improve flavors, prevent microbial growth and enzymatic activity, as well as guarantee a characteristic texture and flavor in the food (5, 6). In Europe, around 75% of the salt consumed comes from processed foods, of which 20% is derived from meat products (7). Spain has one of the highest consumption and production rates of meat products, which justifies the need to substitute or reduce the salt content in this food.

Raw cured sausages are meat derivatives in which their consumer acceptability is mainly due to the fundamental role that salt has on their microbiological, physicochemical, and sensory quality (8). Using NaCl to produce sausages is of vital importance, since it reduces the water activity of the food, thus preventing the development of microorganisms capable of altering the quality of the final product (9). In addition, NaCl has a protein solubilisation effect due to the increase in ionic strength and its subsequent gelation and binding of the particles that make up the mass, until reaching a suitable consistency and texture (10). Salt also conditions the biochemical and enzymatic reactions that take place during the maturation of cured raw sausages, affecting the aroma of the product, besides partially fragmenting the proteins that lead to the release of non-protein nitrogenous compounds, affecting the pH, flavor, and aroma of the sausage (11).

Given the functions that NaCl performs on cured raw sausages, reducing its concentration in this meat product is especially difficult. An alternative to salt reduction is the partial substitution of NaCl with other salts or ingredients (KCl, $CaCl_2$, $MgCl_2$, K-lactate, and glycine, among others) that provide the same technological functions, while maintaining their sensory characteristics, obtaining products that represent an improvement in their nutritional properties (12). The substitutions of NaCl using potassium salts are the most implemented in the reduction of Na. Some researchers studied the effect of reducing NaCl, up to 50%, by using K salts, without producing unfavorable changes in the sensory and microbiological characteristics in sausages (13). Using KCl has been limited mainly by its bitter-metallic taste, but studies have shown that the combination of KCl with NaCl of up to 50% does not detect variations in taste and texture of cooked ham (14), nor in sausages and pork loin (15), agreeing with other studies (16). Some authors confirm that the partial substitution of NaCl by KCl seems to be the best alternative to reduce the sodium content in meat products, since both salts have similar properties and the consumption of K has not been related to developing high blood pressure and cardiovascular diseases (17). Therefore, the partial substitution of NaCl by KCl has been the most common strategy to reduce the salt content in manufactured meat derivatives, but usually with unsatisfactory results. There is also no evidence of the partial substitution of NaCl by KCl in the production of Iberian chorizo, a raw meat derivative cured with high consumption in Spain.

Sea salt an alternative used in the culinary preparation of some foods, as a substitute for NaCl, providing sensory characteristics highly appreciated by the consumer, with greater amounts of minerals and trace elements than NaCl (18). Recently, seawater substitutes with a similar composition have been developed, which solve the inconveniences of treating and transporting seawater. No evidence shows using a combination of salts (seawater substitute) to replace NaCl in the production of cured sausages, nor have studies investigated the reduction of NaCl in Iberian chorizo using KCl as an alternative. This combination of salts, besides reducing the Na content and providing genuine sensory characteristics, increases the concentration of other minerals, allowing the inclusion of some nutritional and health claims in food on its labeling, which are included in the Regulation (CE) 1924/2006 of the European Parliament and of the Council, as "reduced sodium/salt content."

The objective of this study is to develop an Iberian chorizo with better nutritional properties (reduced Na content and greater contribution of other minerals), maintaining or even improving its hygienic quality, sanitary, and organoleptic properties, using a combination of mineral salts, compared to other chorizo produced with KCl and NaCl.

MATERIALS AND METHODS

Samples

Three batches of 12 Iberian chorizos were made in the pilot plant of a Spanish meat company (Treatment 1: CTRL, four samples; Treatment 2: KCl, four samples and Treatment 3: SC, four samples). **Table 1** includes the ingredients used for each formulation (some of them confidential by the meat company). Treatment 1 is the control formulation, with 100% NaCl; Treatment 2, the KCl formulation has 31% of the NaCl replaced by KCl (31% KCl + 69% NaCl); also, a masking aroma was incorporated to avoid possible bitter flavors derived from KCl; furthermore Treatment 3, the SC formulation, where 100% NaCl has been replaced with 97% of combined mineral salts (97% SC + 3% NaCl) (Winbi®; whose Na content is 25% lower than the control). The composition of the combined mineral salts (Winbi®) is shown in **Table 2**. As a starter, a mixture of *Staphylococcus xylosus* and *Pediococcus pentosaceus*, supplied by the Oleica company (Guaro, Malaga, Spain) was used. The manufacturing process was the same for all batches. First, the meat and fat were minced in a mincer with 60 and 10 mm orifice plates. Second, the rest of the ingredients were incorporated into the mass, mixing them using a vacuum mixer for approximately 4 min. Subsequently, each mass was stuffed into collagen casings of caliber 47, forming pieces with an average weight of 695 g. After draining the pieces, they were transferred to a dryer with a temperature of 5 ± 2°C and 70–85% of relative humidity (RH), where they remained for 21 days. They were then moved to a cellar with a controlled temperature between 12 and 5°C

TABLE 1 | Composition of the different formulations used in the production of Iberian chorizo, expressed as g/Kg of meat.

Ingredients	CTRL	KCl	SC
Paprika	14.31	14.31	14.31
Garlic paste	1.67	1.67	1.67
Oregano	0.14	0.14	0.14
Additive[a]	9.54	9.54	9.54
Nitrifying salt	1.43	1.43	1.43
NaCl	19.08	7.15	-
Salts combined	-	-	18.49
NaCl + KCl + Aroma	-	11.92	-

[a]*Monosodium glutamate and aroma masker for taste.*

TABLE 2 | Composition of salts combined Winbi®.

Ingredients	Minerals	100 g	By unit (1.2 g)
Salt	-	73.7 g	0.9 g
Sodium chloride	Ion Sodium (Na^+)	29.49 g	0.35 g
Magnesium sulfate	Ion Magnesium (Mg^{2+})	2,976 mg	35.71 mg
Magnesium chloride	Ion Chloride (Cl-)	50,849 mg	610.19 mg
Natural sodium sulfate	Ion Sulfate (SO_4^{2-})	7,891 mg	94.69 mg
Calcium chloride	Ion Calcium (Ca^{2+})	1,091 mg	13.09 mg
Potassium chloride	Ion Potassium (K^+)	1,364 mg	16.37 mg
Sodium bicarbonate	Ion Bicarbonate (HCO^{3-})	1,017 mg	12.20 mg

and RH around 70–85% for 27 days. Finally, the chorizos were stored in a natural cellar until the end of their curing, 30 days later, for which the total maturation time was 57 days. To conduct the analyses, first, aseptically, the sample was taken for microbiological analysis. Subsequently, the rest of the chorizo was crushed, where a first part was used to determine moisture, pH, and a_w, the remaining was frozen until the moment of use.

Physicochemical Analysis, Proteolysis, and Mineral Composition

Weight loss was determined by weighing each sample, in triplicate, throughout the entire curing period (80 days). The results were expressed as a percentage of weight loss regarding the initial weight of the whole product.

The moisture, protein, fat, and ash content were determined according to the methods 950.468, 981.10, 920.153, and 960.39(b), respectively, established by the AOAC. All tests were performed in triplicate. The sodium content was determined in triplicate for each formulation using an inductively coupled plasma atomic emission spectrophotometer (ICP-AES Ultima 2, Horiba Jobin Yvon, Milan, Italy). The amount of salt was estimated considering all the Na was in NaCl. The pH was measured using a HI 99163 pH meter (Hanna Instruments Inc., Hoonsocket, Dakota del Sur, USA) as established in the ISO 2917:1999 standard (19). For this, 10 g of each sample with distilled water in a 1:10 ratio in triplicate. The a_w measurement was performed, in triplicate, with a Novasina meter (LabSwift, Metrohm AG, Madrid, Spain) following the AOAC 978.18 method (20).

Non-protein nitrogen (NPN) was determined using the Kjeldahl method, after protein precipitation, using 12.5% trichloroacetic acid (TCA) (21). The results were expressed as mean values in g of nitrogen/kg of dry matter. The proteolysis index (PI) was calculated as the percentage ratio between NPN and total nitrogen (TN) (22).

Mineral determination was conducted using an ICP-AES Ultima 2 (Horiba Jobin Yvon, Milan, Italy). For this, 0.5 g of crushed Iberian chorizo was mineralised in a microwave system (Milestone 1200, FVK, Bergamo, Italy) using 3 mL of nitric acid (Romil Ltd., Cambridge, United Kingdom) and 0.5 mL of Suprapur 30% hydrogen peroxide (Merck, Germany). The samples were diluted with 50 mL of milliQ water and read at different wavelengths: Na (589.59 nm), K (766.49 nm), Ca (393.36 nm), Mg (279.55 nm), P (213.61 nm), Fe (238.20 nm), Cu (346 nm), Mn (540 nm), Zn (213.85 nm), and B (532 nm) (23). The content of each mineral was expressed in mg/100 g of meat.

Instrumental Analysis

Color Measurement

The color was determined using a Color Flex spectrocolorimeter [Hunter Associates Laboratory Inc., Reston, VA, USA (illuminant D65 and a visual angle of 10°)]. The established color parameters were: L*, a*, b*, C*, and H*. Three readings were made per formulation in different slices of the Iberian chorizo at the end of the curing (24).

Texture Profile Analysis (TPA)

Three meat pieces of 2.5 cm of thickness were cut and were subjected to a texture analysis using a Texture Analyzer (TAXT2, Stable Micro System, UK), at 25°C and under the following study conditions: an activation load of 0.44 N, pre-test speed of 2 mm/s, post-test speed of 5 mm/s, distance of 8 mm and a force of 5 g. The texture parameters obtain were hardness, adhesiveness, cohesiveness, elasticity, gumminess, and chewiness.

Sensory Analysis

The study was conducted in a standard room equipped with individual cabins according to this method (25). The panel of tasters comprised 60 untrained people who were given a questionnaire, along with the sliced samples to be tested, which were coded with three random numbers. The sliced samples were served at 25°C and arranged on plates. An acceptance test was performed using a 5-point hedonic scale, considering the rating of 5 (I like it very much) and 1 (I dislike it very much). The sensory attributes established for this study were: color, odor, salty taste, texture, and global assessment. The ethics committee of the Universidad Católica San Antonio does not believe that a declaration of ethics is appropriate for the development of the sensory analysis of this product, as it complied with all food safety requirements.

Microbiological Analysis

Twenty-five grams of sausage sample were homogenized with 225 mL of peptone water (Scharlau Chemie S.A., Barcelona,

Spain) in a Stomacher LabBlender 400 (Seward Medical, London, UK) for 1 min. Dilutions were made from the resulting suspension using peptone water. The quantification of *Salmonella* spp and *Shigella* bacteria were carried out by sowing 0.1 mL of each dilution on XLD agar (Scharlau Chemie S.A., Barcelona, Spain), and *Listeria monocytogenes* was sowed on Oxford agar (Scharlau Chemie S.A., Barcelona, Spain). For the differentiation of species of the genus *Staphylococcus*, 1 mL of the dilutions was added in Baird Parker Agar (Charlau Chemie S.A., Barcelona, Spain). Each dilution was seeded in triplicate for both media and the plates were incubated at 30°C/24 h, for *L. monocytogenes*, and at 37°C/24 h for *Salmonella spp*, *Shigella,* and *Staphylococcus aureus*. The results were expressed as colony-forming units per gram of Iberian chorizo (CFU/g).

Statistical Analysis

The determinations, except for the sensory analysis, were conducted in triplicate and the results were expressed as the mean and standard deviation. For the sensory analysis only one sample was used for each formulation, so the final scores were averaged over all the panelists. Analysis of variance using a one-way ANOVA procedure and Tukey's test were performed to determine the effect of the different formulations (CTRL, sodium chloride; KCl, potassium chloride; and SC, combined salts) on all the parameters studied in Iberian chorizo at the end of the curing period. The statistical differences were given when $P < 0.05$. Statistical analysis was conducted using the SPSS version 21.0 software package (IBM Corporation, Armonk, NY, USA).

RESULTS AND DISCUSSION

Physicochemical Analysis, Proteolysis, and Mineral Composition of Iberian Chorizo at the End of Its Curing

Table 3 shows the results obtained from the nutritional composition, physicochemical parameters, and proteolysis of the three batches of Iberian chorizo (CTRL, KCl, and SC). The moisture content of cured meat products is important as it influences their stability and safety. Slight changes in moisture can change the texture and acceptability of the product (26). The chorizos made with the combined salts (SC) or with the partial substitution of NaCl for KCl (KCl), presented lower moisture values than those made with common salt (CTRL). These same results were obtained in an investigation whose objective was to replace NaCl with other salts (replacement, 1.9%), including KCl, to prepare salamis, where the moisture content was higher in salamis elaborated with NaCl than those with KCl (27). These moisture differences may be because the mixtures of salts, mainly formed by KCl, penetrate the meat easier, thus hindering the exit of water from inside the meat (28). This variation could also be justified based on the different a_w values presented by NaCl and KCl, since the a_w of a saturated solution of NaCl at 25°C is 0.753 and that of KCl is 0.843. Thus, when the air from the dryers maintains the a_w of the sausage surface between 0.753 and 0.843, the samples formed only by KCl will have a lower water content on their surface than the samples made with NaCl.

TABLE 3 | Nutrition composition, physicochemical parameters, and proteolysis measure of Iberian chorizo at the end of the curing processing.

Nutrition Composition[1]	CTRL	KCl	SC	*P*-value
Moisture	$19.42^b \pm 0.32$	$16.81^a \pm 0.39$	$17.42^a \pm 0.40$	0.000344
Protein	$28.68^a \pm 1.13$	$31.84^a \pm 0.53$	$31.96^a \pm 3.77$	0.220577
Fat	$35.98^a \pm 3.80$	$34.40^a \pm 2.31$	$32.03^a \pm 3.01$	0.355542
Ash	$6.71^a \pm 0.32$	$6.82^a \pm 0.05$	$6.58^a \pm 0.45$	0.671721
Salt	$3.24^b \pm 0.04$	$2.41^a \pm 0.24$	$2.36^a \pm 0.01$	0.000460
Physicochemical parameters[2]				
pH	$5.25^a \pm 0.02$	$5.4^a \pm 0.15$	$5.2^a \pm 0.10$	0.053
a_w	$0.881^b \pm 0.003$	$0.875^a \pm 0.002$	$0.873^a \pm 0.002$	0.0041
Proteolysis				
Non-protein nitrogen (g nitrogen/dry extract)	$0.08^b \pm 0.01$	$0.14^a \pm 0.01$	$0.14^a \pm 0.00$	0.005578
Proteolysis index	$1.69^a \pm 0.01$	$2.73^b \pm 0.01$	$2.64^b \pm 0.02$	0.0081

Values are mean ± SD (n = 3). One-way ANOVA. Different letters in the same row indicate a significant different ($P < 0.05$) between the samples SC (salt combination), KCl (potassium chloride), and NaCl (sodium chloride, common salt).
Treatment I: CTRL (100% NaCl); Treatment II: KCl (31% NaCl + 69% KCl); Treatment III: SC (3% NaCl + 97% SC).
[1] *g/100 g of Iberian chorizo.*
[2] *dimensionless.*
a_w, *water activity.*

With a lower water content on the food surface, the weight loss will be greater and the moisture content lower (29). The greater loss of water in SC and KCl is corroborated with the product weight loss during processing (**Table 4**). Significant differences were observed in weight loss between the different formulations from the 15th day of curing, observing higher values in KCl (17.99%) and SC (17.01%) sausages, regarding CTRL (14.88%). It was observed that the decrease during the entire ripening process was lower in chorizo made with NaCl (CTRL), with values of 34.12, 40.02, and 38.21% for the CTRL, KCl (formulation after 80 days), and SC, respectively.

No significant differences were observed in the content of protein, fat, and ash ($P > 0.05$). Nutritionally, in relation to proteins, it is shown that, for the three batches of chorizo, the nutritional declaration "high protein content" could be made, since proteins provide over 20% of the energy value of the food in these cases (30). In addition, CTRL, KCl, and SC can make the approved health claims that protein contributes to building muscle mass, maintaining muscle mass, and maintaining normal bones (i.e., bone calcium levels and bone density) (31).

The NaCl concentration was higher in CTRL (3.24/100 g) than in KCl and SC. Thus, the chorizo made with the combination of mineral salts (SC) obtained the greatest reduction in NaCl (2.36/100 g), followed by KCl (2.41/100 g). These results coincide with those obtained by Horita et al. who achieved a similar compatibility relationship by replacing NaCl with 50 and 75% KCl to prepare "mortadela" (a dry-cured luncheon meat) (32). Ibáñez et al. achieved Na reductions similar to those obtained

TABLE 4 | Weight loss of Iberian chorizo during the curing processing.

Days	CTRL	KCl	SC	*P*-value
0	0	0	0	
5	$7.50^{a} \pm 1.11$	$8.08^{a} \pm 1.11$	$8.10^{a} \pm 0.88$	0.81
10	$12.10^{a} \pm 1.01$	$14.05^{a} \pm 1.66$	$13.77^{a} \pm 1.80$	0.0650
15	$14.88^{b} \pm 2.55$	$17.99^{a} \pm 1.22$	$17.01^{a} \pm 2.01$	0.0475
25	$17.44^{b} \pm 1.88$	$23.22^{a} \pm 3.40$	$22.47^{a} \pm 2.40$	0.0423
30	$21.02^{b} \pm 1.51$	$26.02^{a} \pm 2.80$	$24.98^{a} \pm 3.40$	0.0404
35	$23.44^{c} \pm 2.22$	$28.44^{a} \pm 3.55$	$26.80^{b} \pm 2.11$	0.0338
45	$25.45^{c} \pm 1.22$	$31.01^{a} \pm 2.01$	$28.23^{b} \pm 1.25$	0.0465
50	$27.12^{c} \pm 2.01$	$34.20^{a} \pm 2.11$	$31.54^{b} \pm 2.41$	0.0275
60	$30.02^{c} \pm 1.22$	$36.44^{a} \pm 1.88$	$34.24^{b} \pm 0.88$	0.0350
70	$33.45^{b} \pm 1.15$	$39.14^{a} \pm 0.88$	$37.44^{a} \pm 2.22$	0.0175
80	$34.12^{b} \pm 1.15$	$40.02^{a} \pm 0.80$	$38.21^{a} \pm 0.58$	0.0458

Values are given as mean ± SD (n = 3). One-way ANOVA. Different letters in the same row indicate a significant different (P < 0.05) between the samples SC (salt combination), KCl (potassium chloride) and NaCl (sodium chloride, common salt).
Treatment I: CTRL (100% NaCl); Treatment II: KCl (31% NaCl + 69% KCl); Treatment III: SC (3% NaCl + 97% SC).
The results are expressed as percentage weight loss (%).

TABLE 5 | Mineral composition of Iberian chorizo at the end of curing processing.

	CTRL[1]	KCl[1]	SC[1]	*P*-value	RDA[2]
Na	$1{,}280^{b} \pm 17.56$	$950^{a} \pm 96.25$	$930^{a} \pm 4.23$	0.000000	0.006
K	$620^{a} \pm 35.63$	$960^{b} \pm 16.64$	$640^{a} \pm 42.17$	0.000024	2000
Ca	$20^{a} \pm 1.41$	$30^{a} \pm 1.75$	$50^{b} \pm 7.73$	0.001483	800
Mg	$30^{a} \pm 1.00$	$40^{a} \pm 1.45$	$90^{b} \pm 1.78$	0.000000	375
P	$240^{a} \pm 12.73$	$280^{a} \pm 3.75$	$270^{a} \pm 19.33$	0.064915	700
Fe	$4.430^{a} \pm 0.14$	$3.810^{a} \pm 0.12$	$4.250^{a} \pm 0.14$	0.805024	14
Cu	$0.292^{a} \pm 0.00$	$0.332^{a} \pm 0.01$	$0.220^{a} \pm 0.00$	0.091806	1
Mn	$0.198^{a} \pm 0.00$	$0.095^{a} \pm 0.00$	$0.190^{a} \pm 0.00$	0.550735	2
Zn	$4.590^{b} \pm 0.01$	$5.550^{a} \pm 0.02$	$5.280^{a} \pm 0.04$	0.014857	10
B	$0.047^{a} \pm 0.00$	$0.043^{a} \pm 0.00$	$0.043^{a} \pm 0.00$	0.932280	Not declared

Values are mean ± SD (n = 3). One-way ANOVA. Different letters in the same row indicate a significant different (P < 0.05) between the samples: CTRL (sodium chloride, common salt), KCl (potassium chloride), and SC (salt combination).
Na, sodium; K, potassium; Ca, calcium; Mg, magnesium; P, phosphorus; Fe, iron; Cu, copper; Mn, manganese; Zn, zinc; B, boron.
Treatment I: CTRL (100% NaCl); Treatment II: KCl (31% NaCl + 69% KCl); Treatment III: SC (3% NaCl + 97% SC).
[1]*Data are expressed in mg/100 g.*
[2]*RDA: Recommended Daily Allowances of the minerals.*

in this study, by partially replacing NaCl with KCl in fermented sausages (33).

The pH and a_w are essential factors that guarantee the stability and safety of the sausages. Regarding pH, no significant differences ($P > 0.05$) were observed between the three formulations used to prepare Iberian chorizos (**Table 3**), agreeing with what was observed in manufacturing a typical Italian salami reduced in NaCl (23). Regarding a_w, the values obtained in all chorizos were considered normal for these products, and adequately favor their storage and safety. In **Table 3**, slightly higher a_w values can be seen in the CTRL chorizo, probably due to their higher moisture content (19.42%) than the other formulations (KCl, 16.81% and SC, 17.42%). In addition, adding solutes, such as mineral salts, helps to reduce the value of a_w, since when hydrated, the availability of water is reduced (34). Therefore, the chorizo made with the SC formulation has a lower a_w (0.873). Similar studies presented results approximate to those of this study, like Corral and Flores (6). These authors observed a decrease in a_w when replacing 50% of NaCl with KCl, besides concluding the need to search for other ingredients that, when combined with KCl, achieve greater substitution without causing alterations, especially sensory. However, another study conducted on fermented sausages shows that the highest a_w was developed in sausages made with the highest percentage of NaCl, as occurs in this study (6).

The NPN and the PI were lower in CTRL chorizos than in KCl and SC chorizos (**Table 3**). Some authors have observed that the activity of proteases (calpains and cathepsins) is diminished with the increase of the NaCl content, which justifies the lower PI in CTRL chorizos (21, 35, 36). NaCl has been shown to induce an inhibitory effect on the activity of cathepsins B and B + L. In cured loin some observed that the partial replacement of 50% of NaCl with KCl produces a higher activity of cathepsins B and B + L and, an increase in proteolysis, explaining this higher content of NPN in KCl and SC chorizos (17).

No references have been found on proteolysis in Iberian chorizo, and the values observed in other cured fermented products being highly variable, depending on the product and production process. Thus, in dry fermented sausages, NPN values of 8.8% were observed (37) and in dry sausage from Painho de Portalegre values were 0.85% (38). Furthermore, no studies have evaluated the effect of the substitution of NaCl on the NPN in cured raw sausages. In cured loin, some have also observed that with lower NaCl content the concentration of NPN was higher (21). In cured raw sausages only the effect of NaCl on free amino acids has been studied. Thus, an increase in the content of free amino acids (increased proteolysis) was developed in salamis in which NaCl was substituted with $MgCl_2$ (27). Samples with a 50% reduction in NaCl showed a higher content of the amino acids Arg, Glu, His, Val, Cys, Lys, and Trp. Whereas, the samples formed by $CaCl_2$ had a higher content of the amino acids Asp, Thr, Ala, Met, Leu, Ile, and Phe. The replacement of up to 50% of NaCl with KCl, in the salting stage to prepare cured loins, did not affect proteolysis (39).

The mineral content of the Iberian chorizo made with different formulations is shown in **Table 5**. The Na content decreased significantly with the reduction of the NaCl content, in the different formulations, up to 27.24% (KCl) and 25.71% (SC) compared to the CTRL. Therefore, according to Regulation (EC) 1924/2006, the nutritional declaration "reduced salt/sodium content" can be made on the labeling, presentation, and advertising of SC and KCl chorizos; complying with the conditions of use that the reduction of salt/sodium is at least 25%, compared to a similar product (CTRL). Simultaneously, it enables the SC and KCl chorizos to make the health claim "Reducing consumption of sodium contributes to the maintenance of normal blood pressure."

The K content of sausages made with the KCl formulation is higher (960 mg/100 g) than the CTRL (620 mg/100 g) and SC (640 mg/100 g), since the partial substitution of Na with K provides the food with a higher content of this mineral. Lorenzo et al. studied the elaboration of a cured pork shoulder using different salts as a replacement for NaCl, obtaining results like this study, as the partial replacement of Na with other salts (KCl 50% + NaCl 50%) reduced the Na content and increased K and Mg, compared to the control (100% NaCl) (40). Zanardi et al. obtained similar values in terms of K content. The salami formulated with a low concentration of NaCl (13.5 g/kg) presented a higher content of K (948 mg/100 g) than the control salami (530 mg/100 g) (23).

From a nutritional point of view, these results indicate that the CTRL chorizo contains 31% of the nutrient reference values (NRV) per 100 g, similar results are obtained for the K content in SC chorizo (32% of the NRV per 100 g). Whereas, the chorizo formulated with KCl contains 48% of the NRV per 100 g. Therefore, the CTRL, SC, and KCl chorizos can claim on their labeling the nutritional claims "source of potassium" and "high potassium" (41). In addition, the three samples analyzed as "source of potassium," can make healthy statements on their labeling that "potassium contributes to normal functioning of the nervous system," and "potassium contributes to the maintenance of normal blood pressure" (41). This last statement, for the SC and KCl Iberian chorizos, implies a possible synergistic action in relation to the cardiovascular system, together with the reduction in Na content. However, despite the K content in the KCl chorizo being significantly higher than in the CTRL and SC, for the purposes of statements there are no differences, and the consumer can verify this difference in content through the value reflected in the nutritional information of the product; where vitamins and minerals must be indicated in absolute amounts and %NRV for every 100 g or 100 mL of product.

Regarding Ca, the SC chorizos, present higher values (50 mg/100 g), producing an increase of 110 and 179% compared to KCl (30 mg/100 g) and CTRL (20 mg/100 g), respectively. The SC samples showed high Mg values (90 mg/100 g), compared to KCl (40 mg/100 g) and CTRL (30 mg/100 g). For both minerals (Ca and Mg) no significant differences were observed between the CTRL chorizos and those formulated with KCl. This may be due to the difficulty of divalent cations to penetrate inside the muscle (28). Another study conducted by Fieira et al. did not find significant differences in the content of minerals such as Ca and Mg in the elaboration of salami using different chloride salts (NaCl, KCl, $CaCl_2$, and $MgCl_2$) (42).

The NRV of Ca is 800 mg/100 g (41), thus the results obtained for Ca for the CTRL, KCl, and SC chorizos did not reach a significant amount (2.5, 3.75, and 6.25%, respectively); therefore, the nutrition claim "source of calcium" cannot be made, nor any health claims relating to this mineral. Regarding Mg, its NRV is 375 mg/100 g (41); here, results show that the CTRL chorizo contains 30 mg/100 g, the KCl chorizo contains 40 mg/100 g, and the SC chorizo contains 90 mg/100 g, as seen in **Table 5**; this represents 8, 10.66, and 24% of NRV, respectively. Therefore, only Iberian chorizo made with the SC formulation could make the nutritional claim "source of magnesium" on its labeling, and health claims authorized for this mineral. In relation to the products studied, it is worth highlighting the contribution of Mg to the electrolyte balance, performing an adjuvant action derived from the decrease in Na and increase in K in the formulation of SC and KCl chorizos.

The SC and KCl chorizos present higher *P*-values than the CTRL chorizo, but no significant differences were observed between the different formulations used (**Table 5**). Zanardi et al. did not observe significant differences of this mineral on the different formulations used in manufacturing an Italian-type salami (23). From a nutritional viewpoint, the NRV for P is 700 mg (41). According to the results obtained in this study, all chorizo samples could include in their labeling these nutritional declarations: "source of phosphorus" and "high phosphorus," as for all formulations the calculation of the NRV is above that stipulated 15%, specifically: 34.28% for CTRL, 38.57% for SC, and 40% for KCl chorizos. Consequently, health claims authorized for P could also appear on its labeling (31).

In relation to the content of Fe, Cu, and Mn, in the Iberian chorizo samples, the results show similar figures without observing significant differences ($P > 0.05$) between the different formulations used. After performing the nutritional calculations based on the NRVs, the Iberian CTRL and SC chorizos can make the nutritional claims "source of iron" and "high iron," while the chorizo made with the KCl formulation meets the conditions use only of the nutrition declaration "source of iron." However, the three samples could make health claims authorized for the Fe (31). Nutritionally, the results for Cu represent a NRV of 33.2, 22.2, and 29.2% for the CTRL, SC, and KCl chorizos, respectively. According to these values, all samples can make the nutritional declaration of "source of cooper," while the KCl formulation could also make the declaration of "high copper" on its labeling (41); thus authorized health claims can be made on all chorizo labeling (31). With Mn, in all three cases, there are discrete amount changes that do not reach significant values to make nutritional or health claims on their labeling.

With Zn, higher values are observed in the KCl (5.55 mg/100 g) and SC formulations (5.28 mg/100 g) compared to the CTRL (4.59 mg/100 g), which represents 55.5, 52.8, and 45.9% of the NRV (10 mg), respectively. Prepared salami, with different formulations, showed an increase in the Zn content, when the formulation comprised low concentrations of NaCl (23); corroborating results in this study. In all the samples the contribution of Zn is remarkable, which can be reflected in their labeling of the nutritional declarations "source of zinc" and "high zinc," as well as the health claims authorized for this mineral (31). Of these statements, given the characteristics of the object of this study, it is worth highlighting those relating to "Zinc contributes to normal metabolism of fatty acids," and "Zinc contributes to the protection of cells from oxidative stress."

Finally, the results related to B show similar values, without observing significant differences between the different formulations (**Table 5**). There is no established NRV for this mineral, nor is there any authorized nutrition or health claim.

TABLE 6 | Instrumental color measure, TPA, and sensory attributes and consumer acceptability of Iberian chorizo at the end of the curing processing.

Color parameters	CTRL	KCl	SC	*P*-value
L*	$37.86^{c} \pm 0.98$	$30.63^{a} \pm 0.85$	$33.25^{b} \pm 0.79$	0.000166
a*	$27.75^{a} \pm 1.79$	$26.93^{a} \pm 0.53$	$24.15^{a} \pm 2.74$	0.131291
b*	$22.09^{a} \pm 2.73$	$21.29^{a} \pm 0.21$	$15.88^{b} \pm 0.70$	0.006826
C*	$35.47^{a} \pm 3.10$	$34.33^{a} \pm 0.28$	$28.91^{b} \pm 2.66$	0.030975
h	$38.43^{a} \pm 1.61$	$38.34^{a} \pm 0.84$	$33.46^{b} \pm 1.96$	0.011909
Texture Profile Analysis				
Hardness	$35.55^{a} \pm 9.90$	$82.62^{b} \pm 8.88$	$48.37^{a} \pm 14.32$	0.003733
Adhesiveness	$0.60^{a} \pm 0.14$	$0.50^{a} \pm 0.14$	$0.60^{a} \pm 0.56$	0.946910
Cohesiveness	$0.38^{ab} \pm 0.007$	$0.40^{b} \pm 0.02$	$0.33^{a} \pm 0.01$	0.403560
Elasticity	$1.80^{a} \pm 0.08$	$1.83^{a} \pm 0.10$	$1.85^{a} \pm 0.12$	0.900776
Gumminess	$17.56^{ab} \pm 2.38$	$32.94^{b} \pm 5.11$	$15.59^{a} \pm 2.73$	0.044105
Chewiness	$18.66^{a} \pm 4.08$	$60.35^{b} \pm 12.79$	$29.44^{ab} \pm 3.39$	0.040178
Sensory Attributes				
Appearance	$3.46^{a} \pm 0.97$	$3.66^{ab} \pm 1.12$	$4.04^{a} \pm 1.08$	0.023381
Odor	$3.52^{a} \pm 1.05$	$3.78^{a} \pm 1.01$	$3.78^{a} \pm 1.11$	0.370412
Texture	$3.38^{a} \pm 1.06$	$3.34^{a} \pm 1.08$	$3.82^{b} \pm 1.00$	0.043171
Salty Taste	$3.47^{a} \pm 0.86$	$3.66^{ab} \pm 0.93$	$3.84^{b} \pm 0.89$	0.135018
Global Taste	$3.57^{a} \pm 0.95$	$3.67^{a} \pm 0.96$	$4.28^{b} \pm 0.92$	0.000474
Consumer acceptability	$3.59^{a} \pm 0.95$	$3.77^{a} \pm 0.88$	$4.22^{b} \pm 1.05$	0.004801

Values are mean ± SD (n = 3). One-way ANOVA. Different letters in the same row indicate a significant different (P < 0.05) between the samples.
CTRL (sodium chloride, common salt), KCl (potassium chloride) and SC (salt combination).
Treatment I: CTRL (100% NaCl); Treatment II: KCl (31% NaCl + 69% KCl); Treatment III: SC (3% NaCl + 97% SC).
L, lightness; a*, redness; b* yellowness; C*, saturation; h, tone.*
Hardness (N, Newton); Adhesiveness (mJ, millijoules); Elasticity (mm, millimeters); Gumminess (N); Chewiness (mJ).

Instrumental Analysis of the Iberian Chorizo at the End of Its Curing

Table 6 shows the mean values of the parameters of color, texture, and sensory analysis of the Iberian chorizo. Significant differences ($P < 0.05$) were observed in the color parameters L*, b*, C*, and H* in the different formulations used. The CTRL chorizo showed a higher luminosity value (L*) (37.86) than the KCl (30.63) and SC (33.25) chorizos. Therefore, there has been a darkening of the SC and KCl chorizos, being more evident in the latter.

This color change may be due to the greater loss of water from the product observed during the curing period (43). The chromatic coordinate a* did not show significant differences ($P > 0.05$) between the different formulations used. However, the other color parameters (b*, C*, and H*) were lower in chorizos made with the combination of mineral salts (SC), compared to the CTRL and KCl chorizos. The few color differences between the CTRL, KCl, and SC chorizo, when reducing the NaCl content, is due to the chemical nature and ionic strength provided by the components used as substitutes (44). Although the reduction or replacement of NaCl by other salts develops a loss of the reddish hue typical of the pre-cured mixture. These results agree with those obtained from studying the influence of the replacement of NaCl by KCl to prepare a dry meat sausage (45).

Regarding the results obtained in the TPA, of the different chorizos analyzed (**Table 6**), adhesiveness and elasticity are the only parameters showing no significant differences were observed ($P > 0.05$) between the formulations. However, the KCl chorizo showed higher values of hardness, cohesiveness, gumminess, and chewiness, than the SC chorizo. The increased hardness of KCl chorizo may be related to the percentage of salt used during processing. Previous studies have shown that salt (NaCl) inhibits the activity of cathepsins B and B+L (17). However, the presence of high concentrations of KCl implies a higher activity of these cathepsins, and consequently an inhibition of proteolysis is produced (17). Therefore, in this study, the KCl chorizo presented higher hardness values (82.62 N) than the CTRL (35.55 N) and SC (48.37 N) chorizos. Corral and Flores also observed a decrease in cohesiveness and chewiness in the elaboration of sausage, when the NaCl content was reduced (46). Laranjo et al. also observed significant differences in cohesiveness with samples made with the highest percentage of salt presenting the highest values (47). However, Cittadini et al. found no significant differences in the cohesiveness, gumminess, and chewiness of jerky samples made with different percentages of salts; likewise with those of our study (48).

Microbiological Analysis

No significant differences were observed in the counts of *Salmonella* spp., *Shigella, Staphylococcus,* and *L. monocytogene*s (data not shown) and complied with the limits established by Regulation (EC) 2073/2005, relative to the microbiological criteria applicable to food products.

Slightly acidified fermented sausages often have a pH > 5.3, so one of the safety barriers, acidity, is lost, thus facilitating the development of certain pathogenic microorganisms. In this study, the pH values (**Table 3**) were kept below what was established, so it was not considered a risk factor for microbiological growth. During the chorizo fermentation process, the Enterobacteriaceae counts (*Salmonella*) remained constant. Throughout curing, they did not develop differences in growth (CFU/g) for this microorganism, obtaining an absence in 25 g of chorizo. This result may be because not only NaCl is a total barrier to microbial growth, but it may also influence the addition of Na nitrites in the formulation, since this ingredient can slow bacterial growth (12). Ibáñez et al. obtained similar results, they did not observe significant differences in the analyses conducted on the control sample and on the modified sample (partial substitution of KCl), in the count of *Salmonella* spp., *Staphylococcus aureus*, *Clostridium* sulphite-reducing and *Escherichia coli* (49). In sausages, *S. aureus* is found in low levels (50). NaCl and nitrites have little effect on this microorganism, but under acidic pH and low temperatures its growth is considerably hampered. Another determining factor in the growth of this microorganism is the a_w, as it is vitally important this is kept below 0.955 (51). In the specific case of

this study, the a_w of all the chorizo formulation was below 0.9 (**Table 3**).

Sensory Analysis of the Iberian Chorizo at the End of Its Curing

The results of the sensory analysis of chorizo are shown in **Table 6**. Significant differences are observed in all the sensory attributes studied ($P < 0.05$), except in the odor ($P > 0.05$). The CTRL sample was the worst valued by consumers. This may be because the composition of the food and the release of its aroma affects the release of salivary compounds, the most implicated being: mucins, some enzymes and small molecules such as salts (52). High salivary salts increase the release of flavorings in the oral cavity due to the salty effect, resulting in abnormal and undesirable flavors (53).

This negative assessment, regarding appearance, may be because the CTRL chorizo developed an unwanted colouration. The L^* value in the instrumental measure of color (**Table 6**) showed the CTRL chorizo developed a whitish colouration compared to KCl and SC. Therefore, the consumer preferred chorizos with a darker color. The salty flavor was better valued in the SC chorizo than in the CTRL, thus using other salts did not affect the acceptance of salty.

Cevallos observed a lower hardness and worse texture in pork ham with a partial substitution of NaCl for KCl. However, in this study, the SC chorizos obtained a better texture rating than CTRL and KCl, not observing significant differences between the latter two and agreeing with what was observed in cured loin (17). The texture of the food has a great influence on the chewing process, in particular on the number of chewing cycles, as a hard, dry food requires a greater number of cycles to be fragmented into particles and impregnated by saliva before being swallowed (54). Another study by Horita et al. found no differences in appearance and aroma in "mortadela" made with different chloride salts (32). Regarding the overall flavor and acceptability of the product, the SC chorizo was the best valued by consumers, with the KCl chorizo being the second best valued and then the CTRL. The substitution of salt for other salts has improved its sensory properties, observing significant differences in the SC chorizos, in most attributes studied, compared to the CTRL and KCl. During the oral processing of a food, sensory characteristics are developed, some of them evaluated in this study, due to the release of compounds responsible for them, and which have been previously mentioned. This leads to the perception of the organoleptic properties of the food, contributing significantly to the acceptance of the product by consumers (55), being in this research the SC chorizo the best valued by the consumer panel.

CONCLUSIONS

The partial replacement of NaCl using a mixture of salts (KCl and SC) has a great influence on the moisture content of Iberian chorizo, since the use of salts hinders water loss from the food composition. Using a combination of mineral salts, from seawater, could be considered an alternative in the complete substitution of other chloride salts, since its use has reduced the Na content by up to 30.15%. Using the combined salts (SC) considerably reduces the concentration of salt in the final product, allowing nutritional and health claims for most minerals studied in its labeling. In addition, using low salt content favors the activity of proteases, and increases the proteolytic activity of sausages, thus favoring their sensory characteristics. However, this reduction of NaCl showed a loss of the reddish hue typical of this sausage, making it darker. The partial substitution of NaCl did not negatively affect the sensory characteristics, but also with SC chorizos, they were better valued by the consumer panel.

Finally, using NaCl substitutes does not compromise the stability and safety of the final product, as they meet the required microbiological conditions established in their corresponding regulation. The reformulation conducted in the SC and KCl chorizos give Na values that result in a product that could carry the nutritional claim "reduced sodium/salt content" compared to a similar product. Therefore, it is possible to produce a cured sausage with reduced Na content, by partially substituting it with KCl, or by replacing it with a combination of mineral salts.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LT, ES, and AA: conceptualization. EÁ, BM, and ES: methodology. LT, AA, BM, and ES: investigation. LT and LB-M: data curation. LB-M, LT, and AP: writing-original draft preparation. LT, LB-M, and AP: writing-review and editing. All authors: have read and agree to the published version of the manuscript.

FUNDING

This research was financially supported by project Applied research for the design and development of a new process for reduction of the sodium content in boneless ham from pork (Iberian RIS3MUR).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Nutrition

ORIGINAL RESEARCH
published: 26 March 2021
doi: 10.3389/fnut.2021.620335

Effects of Apple Form on Energy Intake During a Mid-Afternoon Snack: A Preload Paradigm Study in School-Aged Children

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Consuming foods with a form or a texture that requires longer oral processing is a way to decrease food intake. Although this approach is promising for leveraging healthier eating patterns in adults, it has never been explored in children. This study evaluated whether starting a mid-afternoon snack by eating either apple segments or applesauce would modify hunger and subsequent food intake during this meal. Forty-four children (8–10 years old) participated in two videotaped mid-afternoon snacks, during which they received one of the two forms of apple as a food preload followed 10 min later by *ad libitum* consumption of sweetened cottage cheese. They self-reported their level of hunger throughout consumption, and the weight of cottage cheese consumed was determined at the end of the snack. Children's chewing capabilities and eating traits were parent-reported. Eating a raw apple increased oral exposure time and decreased bite size compared to eating applesauce. However, neither the reported hunger nor consecutive food intake were modified. Regardless of the meal, children eating fast had a higher *ad libitum* energy intake. The individual eating rate for the cottage cheese was correlated with the eating rate observed for applesauce but not for apple segments, the latter being associated with children's chewing difficulties. This study suggests that the form of a fruit offered at the start of a mid-afternoon snack does not impact food intake; the findings clearly call for more exploration of satiation mechanisms related to food texture properties among children and indicate the need to consider children's oral processing skills.

Keywords: food texture, preload paradigm, Food Oral Processing, satiation, CEBQ, eating rate, eating behavior, mastication

OPEN ACCESS

Edited by:
Ana Carolina Mosca, University of Udine, Italy

Reviewed by:
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Specialty section:
This article was submitted to Nutrition and Food Science Technology, a section of the journal Frontiers in Nutrition

Received: *22 October 2020*
Accepted: *25 January 2021*
Published: *26 March 2021*

Citation:
Schwartz C, Person O, Szleper E, Nicklaus S and Tournier C (2021) Effects of Apple Form on Energy Intake During a Mid-Afternoon Snack: A Preload Paradigm Study in School-Aged Children. Front. Nutr. 8:620335. doi: 10.3389/fnut.2021.620335

INTRODUCTION

Childhood obesity is a public health concern in all regions of the world: "over 340 million children and adolescents aged 5–19 years old were overweight or obese in 2016" (1). Researchers, governmental policy makers, and food industries have the responsibility to think about new strategies to leverage healthier eating behaviors and decrease energy intake and, in turn, weight gain in children.

In adults, there is strong evidence that food oral processing should be considered as a determinant of food intake. Manipulations of food texture in such a way that decreases bite size and/or increases chewing per bite (and thus oral exposure time) yield a decrease in intake of these

foods (2). It has been suggested that a 20% reduction in eating rate (g/min) is necessary to reach a 10–15% energy intake reduction (3). Thus, a large set of studies investigated the efficiency of changing food form, textural properties, textural complexity, or shape in reducing intake [see many reviews for details: (2, 4–10)]. Large effects are usually observed when the food form is modified (i.e., from liquid to semisolid) (2). Interestingly, changing the food form has also been found to impact the intake of other foods eaten during the same meal (and yet a meal is often a combination of several dishes). In their study, Flood-Obbagy and Rolls (11) used a preload paradigm and tested the effect of starting a meal by consuming apple offered as juice (liquid), juice with added fiber (liquid), applesauce (semisolid), and apple segments (solid) (all forms being matched for energy content, weight, energy density, and ingestion rate) on subsequent *ad libitum* energy intake at the same meal. They observed that apple segments reduced the subsequent food intake more than applesauce or apple juice. This suggests that offering fruit (which is advocated in children) with a different form (raw rather than pureed or as a juice) might be a way to decrease the consumption of other foods offered within the same meal and, in turn, to reduce energy intake. However, although research on the impact of manipulation of food oral processing (though a manipulation of the food texture or the food form) on intake has been widely investigated in adults, very few studies have explored this in children. These studies suggest that the shape and serving size of a vegetable (whole vs. diced carrots) play a role in *ad libitum* intake (12, 13). More research is warranted with child-tailored paradigms, especially on the potential of food manipulations inducing changes in food oral processing for preventing overconsumption during a meal.

Food liking and familiarity are strong drivers of food intake in children (14). Changes in food texture affect acceptance in 3- to 4-year-old children (15) and preference in school-aged children (16). In addition to food liking, food familiarity, and texture acceptance, individual oral processing skills and behavior during a meal are other individual determinants of food intake, particularly through the induced eating rate. Among 4.5-year-old children enrolled in the GUSTO Singaporean cohort, children described as "fast eaters" had a larger average bite size, fewer chews per gram of food, and higher energy intake than children who were "slow eaters" (17, 18). Interestingly, overweight children were more likely to be characterized as "fast eaters" (17). In addition, another study showed that the number of mouthfuls of food/minute observed at 4 years predicted changes in body mass index (BMI) from 4 to 6 years, suggesting that a rapid eating style may be a behavioral marker for the development of childhood obesity (19). Certainly, the development of masticatory efficiency and changes in oral physiology occurring during childhood play a role in the individual eating rate and the ability to cope with more or less complex textured foods. Indeed, between 6 and 10 years, changes in jaw displacements, dentition stage (early mixed dentition), and development of bite force influence the masticatory behavior of children (20). Some children might encounter chewing difficulties when eating harder foods, for example. Pioneering work from Linas and colleagues (21, 22) reported that children presenting early childhood caries (ECC) have more eating difficulties and produce a reduced breakdown of hard foods in the oral cavity (as assessed from the granulometry of their food bolus collected at swallowing). Less information is available about the variability in oral processing behaviors among children with healthy dentition and how this can influence eating rate during a meal. Finally, children's eating rate is associated with their parental reports of food fussiness, food enjoyment, and satiety responsiveness [as evaluated by the Children's Eating Behavior Questionnaire (CEBQ)] (23), suggesting the need to also consider children's appetitive traits when studying their oral processing behavior.

Within this context, our main objective was to probe whether changing the form (semisolid to solid) of a fruit offered at the beginning of a mid-afternoon snack ["goûter," which is a very common practice among children in France (24)] would influence the level of reported hunger and subsequent intake of another food offered within the same mid-afternoon snack (refer here after as snack or meal) in children. A secondary objective was to explore the variability in children's oral processing behaviors when eating the different foods offered during the snack and their potential links with parent reports of children's chewing behavior, chewing difficulties, and appetitive traits. Our hypotheses were as follows: (i) consuming a raw apple preload would increase oral exposure time and subsequently reduce *ad libitum* intake during the snack compared to a pureed apple preload; (ii) high *ad libitum* intake would be related to a high eating rate (i.e., fast eaters are eating more); and (iii) at an individual level, eating rate would be associated with appetite traits and chewing behavior for chewable food.

METHODS

Participants

Children aged between 8 and 10 years old were recruited in 2019 through flyers distributed in schools and afterschool leisure centers and by contacting parents registered in the ChemoSens Platform's PanelSens database [Commission Nationale de l'Informatique et des Libertés (CNIL, n 1148039)] to participate in two mid-afternoon snacks in the laboratory. To be included, children had to attend primary schools (3rd, 4th, and 5th grades), regularly consume a mid-afternoon snack, and be consumers of apple and cottage cheese ("fromage blanc" in French). Exclusion criteria were diagnosed feeding disorder, diabetes, and/or food allergy. The methodology complies with the Declaration of Helsinki and was approved by an ethics committee (Comité de Protection des Personnes Ile-de-France II IDRCB 2019-A00890-57). Written informed consent was obtained from parents prior to the start of the study as well as children's oral assent. Our objective was to recruit a sample of 50 children. In the absence of a similar study in children, we were not able to run a power analysis, so we based our sample size choice on other studies conducted in children [e.g., a study with 40 school-aged children

Abbreviations: BMI, body mass index; VAS, visual analog scale; ED, energy density; EI, energy intake; SR, satiety responsiveness; SE, slowness in eating; EF, enjoyment of food; FR, food responsiveness; FF, food fussiness; CEBQ, Child eating behavior questionnaire; ECC, early childhood caries.

highlighted a significant link between a decrease in liking (for a fruit puree) and a decrease in the level of hunger during mid-afternoon snacks (25)] or in adults (11).

Food Products Offered During the Snack

Two foods regularly consumed by children during mid-afternoon snacks were chosen: apple and sweetened cottage cheese (24, 26). Based on the study by Flood-Obbagy and Rolls (11), two forms of apple were chosen and were matched for energy density (ED) and weight: raw apple segments and applesauce (see **Figure 1**). The applesauce for the whole study was prepared once in the laboratory according to the HACCP regulation for hygiene and safety and was frozen at −18°C and then batch-thawed for each experimental day. Pink Lady® apples were peeled, seeded, cooked, and mixed using a Vorwerk robot (Thermomix®); water was added to compensate for water losses during the process to ensure that apple segments and applesauce were isocaloric (a dry extract analysis showed that the applesauce contained 84.8 ± 0.1% water, and the apple segments contained 83.9 ± 0.2% water). The ED of apple segment Pink Lady® was 54 kcal/100 g. Sweetened (5% *w*/*w*) cottage cheese (Jockey®, Danone; per 100 g: 3 g of fat, 4.4 g of carbohydrate, and 6.9 g of protein) was chosen for the subsequent *ad libitum* meal (**Figure 1**). The ED of the cottage cheese (3.3% of fat, Jockey®, DANONE) was 76 kcal/100 g. This choice was similar to the study by Bouhlal and colleagues (27, 28) in 2- to 3-year-old children, which showed that this food was liked but not too much and would probably then limit overconsumption.

Study Timeline and Measurements

Children participated in two mid-afternoon snacks (at 5:10 pm or 6:10 pm) in the laboratory with a 4–14 days delay in between; each mid-afternoon snack lasted approximately 40 min. Having a mid-afternoon snack is culturally very common for children in France (24), is usually composed of several foods (among which fruit purees are very common with the advent of fruit purees in soft pouches), and represents 17% of daily energy intake in 0–10 year olds (26). The timing was chosen so that children could come to the laboratory after school. This is an average of 2.5 h before the usual dinner time observed in France (29).

We chose a preload paradigm, as it is a design allowing direct observation of intake in a well-controlled environment (30). It consists of a randomized crossover satiation trial with participants eating the preload and then eating a test food *ad libitum*. The delay between the start of the preload and the start of the test food is usually no less than 5 min (30). It was theorized that "up to 15 min after intake the delay is consistent with a meal" and thus reflects satiation effect during the same meal (31). Using this design enables us to directly measure the impact of the preload properties (modification of the food form) on satiation and the consumption of the other food served during the same eating occasion while controlling for potential effects linked to texture acceptance given that the consumption of the food preload is compulsory and that the two preloads are matched for weight. With another possible design, "concurrent evaluation," distinguishing the effect of food texture acceptance from the effect of the food form on consumption is more difficult. The study design is presented in **Figure 1**.

A fixed preload quantity was offered, and the consumption of this entire portion was compulsory, but the eating duration was left to the discretion of the child. Then, after a 10-min delay, cottage cheese was served *ad libitum*. The presentation of the food preload was balanced (i.e., half of the children started the first mid-afternoon snack with apple segments, whereas the other half started with applesauce). The chosen portion was 77 g for the preload [apple segments were served into 10 segments of 7 g; this is equivalent to 16% of usual energy intake from mid-afternoon snacks in 0- to 10-year-old children, as reported by the French INCA3 study (26)]. Portion sizes were defined from piloting tests and corresponded to almost the weight of an apple puree pouch (90 g in general), which would be a relevant portion of what is offered during a mid-afternoon snack meal, in particular with other offered foods. The portion of cottage cheese was 500 g: 400 g was served first in a bowl with a spoon; then, each child could ask for two supplemental portions of 50 g to ensure *ad libitum* consumption (500 g of sweetened cottage cheese is equivalent to 181% of usual energy intake from mid-afternoon snacks). Both meals were organized in a sensory evaluation room; parents were waiting for their children in a room next door. Instructions were given to a group of five–six children collectively by an experimenter at the beginning of the meal, and then children were isolated in individual booths to reduce distraction.

Evaluation of Hunger, Liking, and *ad libitum* Intake

The timeline of the snack was as follows (**Figure 1**): first, children evaluated their initial hunger through a dedicated child-tailored 10-cm visual analog scale (VAS) after being collectively trained to use it. It consists of a visual representation (i.e., cartoon figures, one scale for girls and one for boys) of five levels of hunger varying from "I am not hungry at all. My stomach is completely full, and I cannot eat anymore" to "I am very hungry! My stomach is truly really empty and is rumbling at lot" [see (25, 32) for details]. Then, they ate the apple preload entirely and rated their liking on a dedicated child-tailored 10-cm VAS punctuated by smileys [see (25, 32)] and evaluated again their level of hunger (**Figure 1**). After this, and for 10 min that were strictly controlled, they played an online game based on the principle of "where's Wally?©". By the end of this delay, children were offered cottage cheese. They were free to eat as much as they wanted to until they were no longer hungry. Once they stopped eating, they evaluated their liking for the cottage cheese and their level of hunger. Eaten quantities for all foods (preloads and *ad libitum* foods) were evaluated by weighing the containers before and after consumption (1 g, Soehnle Page, Benfeld, Germany).

Coding of Oral Processing Behaviors

In each booth, children were videotaped to make the description of their oral processing behaviors possible and assess the consumption duration for both preload types. Behaviors were coded using Noldus The Observer® software by a single trained video coder. The coding scheme (see **Table 1**) was inspired by the one used in Fogel and colleagues (17). The validity of the

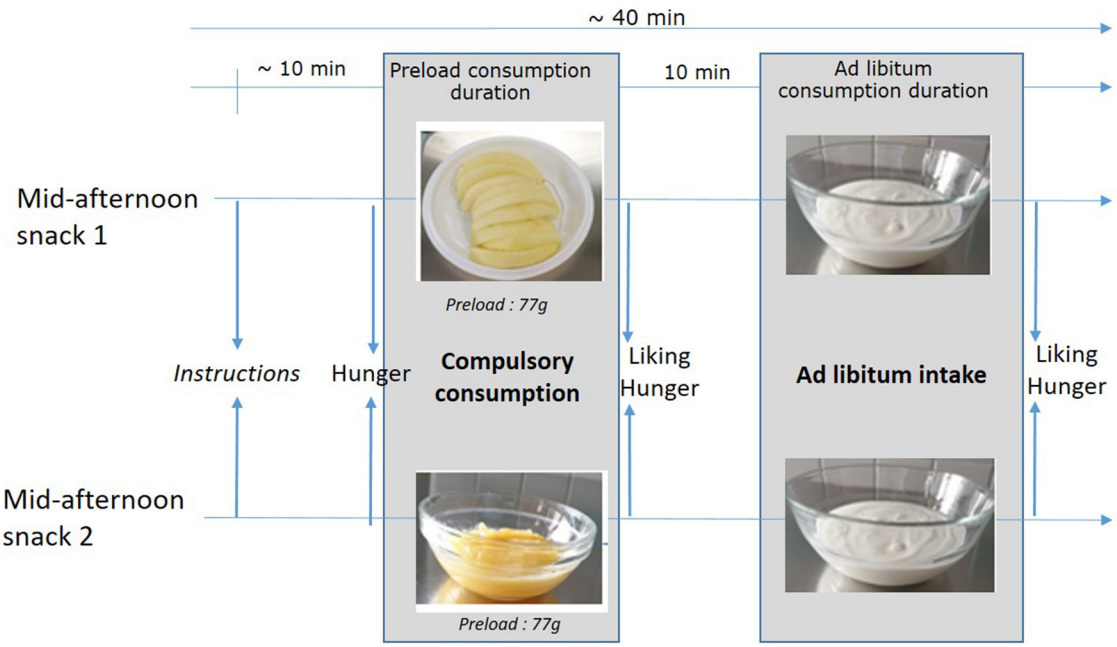

FIGURE 1 | Mid-afternoon snack design (preload and *ad libitum* consumption durations were left to the discretion of the child and are presented in **Table 4**; mid-afternoon snacks starting with apple segments or applesauce were balanced between children).

coding scheme was checked by estimating the agreement with another trained coder (Pearson correlation coefficients for the coded variables: $r = 0.75$ to 0.99) on a subsample of 10 randomly selected videos.

Parental Self-Report of Children's Chewing Behavior and Eating Temperament

Parents were asked to fill out questionnaires to evaluate some facets of their child's eating behavior. Their perception of their child's chewing difficulties and chewing behavior traits was assessed via five items (see **Table 2**). To define the items, we were inspired by previous works (33, 34) but adapted the items for this study to assess only children's difficulties coping with hard/difficult textures and children's chewing behavior and with a limited number of items. Parents answered each item with a three-category answer for each item ("yes," "no," and "I don't know"). Answers "yes" were coded as 1, "no" as 2, and "I do not know" as missing. Two scores were derived (as the mean for the concerned items): one relates to the difficulty of coping with hard/difficult textures (P-noDiff_HardTexture; the higher this score is, the lower the difficulty of coping with hard/difficult textures) and the other describes the extent to which children chew their food before swallowing (P-Chewing; the higher the score, the better the child chews the food before swallowing). The Cronbach's α values were satisfactory for both dimensions [P-noDiff_HardTexture (2 items) = 0.70; P-Chewing (three items) = 0.89].

Children's eating temperaments were assessed with the Child Eating Behavior Questionnaire [CEBQ (35)], and some dimensions only were retained for this analysis: slowness in eating (SE) (e.g., "My child eats slowly"), satiety responsiveness (SR) (e.g., "My child leaves food on his/her plate at the end of a meal" or "My child gets full before his/her meal is finished"), enjoyment of food (EF) (e.g., "My child loves food"), food responsiveness (FR) (for example, "My child is always asking for food"), and food fussiness (FF) (e.g., "My child is difficult to please with meals"), as done by Fogel and colleagues (23). Parents had to indicate how much this was true for their child (from 1: never to 5: always). The score for each dimension is the mean of the different items, and by definition, it ranges from 1 to 5. The Cronbach's α values were satisfactory for the five dimensions (SR = 0.80; SE = 0.79; EF = 0.74; FR = 0.86; FF = 0.90).

Anthropometric Measurements

Children's weight and height were measured in duplicate by a trained experimenter. Children were weighed to the nearest 0.1 kg using a digital scale (PHARO 200, Soehnle, Benfeld, Germany) without shoes. Their length was measured to the nearest 0.1 cm using a stadiometer (TANITA Leicester, Birmingham, UK). Weight and height were transformed into BMI z-scores (BMIz) corrected for age and sex according to the WHO child growth reference for school-aged children and adolescents (36).

Statistical Analysis

SAS 9.3 for Windows (SAS Institute Inc., Cary, NC) was used to perform the analyses. The results are expressed as the means $\pm$ SDs. Significance was set at $p < 0.05$. To check that the food oral processing behaviors and oral exposure time thereof were significantly different between the two types of preload, paired

TABLE 1 | Video-coded oral processing behaviors, methodological parameters, and output variables.

	Definition of behaviors	Coding scheme*	Output variables
1. Consumption duration (segments and applesauce)	Ingestion behavior between the start of the consumption corresponding to the first lip–food contact, when the child closed his mouth after the first bite until the end of the consumption corresponding to the swallowing of the last bite.	State event	Total consumption duration throughout the consumption episode (s): **Duration (s)** The average eating rate was calculated by dividing the grams consumed over the consumption duration: **EatingRate (g/min)**
2. Bite (segments and applesauce) number and duration	Duration from the time when the food enters into the mouth up to the swallows (sequence of biting/eaten mouthfuls and swallows)—this duration does not include breaks (when no food was into the mouth)	State and point event	Number of bites/mouthfuls to eat the entire portion: **Bites (*N*)** Cumulated duration of bites/mouthfuls to eat the entire portion (consumption duration–cumulative duration of breaks): **OralExposureTime (s)** The average bite size was calculated by dividing the grams consumed by the number of bites/mouthfuls to eat the entire portion: **BiteSize (g per bite/mouthful)**
3. Chew or masticatory cycle (segments only)	Up and down movements of the jaws during apple segments consumption. This behavior was coded for 3 out of 10 segments[a]	Point event	Number of up and down movements of the jaws for three apple segments: **MasticatoryCycles (*N*)**

**State events have a duration (e.g., consumption duration), point events do not (e.g., number of chews). [a]For a majority of children, this was done for segments 4, 5, and 6; when necessary due to problems of visibility on the videos, this was done for segments 3, 4, and 5 or 5, 6, and 7.*

Student's *t*-tests were calculated on the oral processing variables. Differences in liking (0–10) between both preloads and cottage cheese after each preload type were also studied (paired *t*-tests).

Impact of Apple Preload Form on Subsequent Hunger and *ad libitum* Intake

Despite the compulsory consumption of the apple preload, some children could not manage to eat it all. A minimal preload consumption of 80% (i.e., at least 62 g of 77 g) was set to analyze the cottage cheese intake data (37). We verified that preload intake was not different between preload forms using an analysis of variance (subject, preload). A mixed model was calculated to evaluate whether the hunger level changed over the session: the dependent variable was the hunger level (0–10), with "subject" as a random factor and with time (three levels: before preload, after preload, and after subsequent *ad libitum* consumption), preload type (two levels: apple segments and applesauce), and the preload type × time interaction as the fixed effects. The mean percentage of hunger decrease due to preload consumption [i.e., (rating score before preload consumption minus rating score after consumption) divided by rating score before preload consumption] and due to cottage cheese consumption (similarly, difference between after preload consumption and after *ad libitum* consumption) were determined and analyzed using ANOVA (subject, preload).

The impact of preload type on cottage cheese *ad libitum* intake (in grams or in kilocalories) was assessed using a mixed model adjusted for initial level of hunger, child age, BMIz, and sex. The correlation between cottage cheese intake and the observed eating rate was assessed using the Kendall correlation coefficient ($p < 0.05$).

Eating Rates: Impact of Food Form and Links With Parental Reports of Chewing Behavior and Eating Temperament

Kendall correlation coefficients were calculated to assess the associations between eating rates observed for the cottage cheese and for the preload eaten during the same snack. The effect of chewing difficulties (P-NoDiff_HardTexture) and chewing activity before swallowing (P-Chewing) on eating rate was assessed using one-way analysis of variance with chewing level as a factor with Student Newman–Keuls *post hoc* analysis. P-NoDiff_HardTexture was obtained by means of two items and therefore has three levels: 1 (the child has difficulties), 1.5 (the child sometimes has difficulties), or 2 (the child has no difficulties). When a significant effect was observed on eating rate, we further explored the effect of chewing difficulties on observed oral processing behaviors (bite size, number of bites, oral exposure time, and number of masticatory cycles) with a Bonferroni adjustment (alpha level of $0.013 = 0.05/4$ per test). Associations between eating rate and CEBQ dimensions (SR, SE, EF, FR, and FF) were explored using Kendall correlation coefficients and Bonferroni adjustment (alpha level of $0.01 = 0.05/5$ per test) to correct for multiple testing.

RESULTS

Participants

Fifty children agreed to participate, but two withdrew before coming to the sessions, and four children did not meet the minimal preload consumption (three children ate less than 80% of the apple segments, and one child ate less than 80% of both apple preloads). Data for 44 children were available (23

TABLE 2 | Parental questionnaire to evaluate their child's chewing difficulty and chewing behavior traits.

	Oui/*Yes* ***Coded as 1***	**Non/*No*** ***Coded as 2***	**Je ne sais pas/** ***I do not know***
1.R (B) Mon enfant prend le temps de bien mâcher ses aliments *My child takes the time to chew his food*	☐	☐	☐
2. (A) Mon enfant refuse de manger certains aliments parce qu'il les trouve trop difficiles à mâcher *My child refuses to eat certain foods because he/she finds them too difficult to chew*	☐ Si oui, lesquels: *If yes, which ones*	☐	☐
3. (B) Lorsqu'il/elle mange, mon enfant a tendance à mettre trop de nourriture/à prendre de trop grosses bouchées, par rapport à la taille de sa bouche *When he/she eats, my child tends to put too much food on his/her fork/spoon or takes too large mouthfuls in relation to the size of his/her mouth*	☐	☐	☐
4. (B) Mon enfant mâche très peu sa nourriture, j'ai l'impression qu'il/elle avale 'tout rond' *My child doesn't chew his/her food much, I have the impression that he/she eats everything whole*	☐	☐	☐
8. (A) J'ai besoin de couper la viande de mon enfant en très petits morceaux pour l'aider à manger *I need to cut my child's meat into very small pieces to help him/her to eat*	☐	☐	☐

Two composite scores were extracted: one relates to the difficulty of coping with hard/difficult textures (P-noDiff_HardTexture; (A) items in the table), whereas the other relates to the fact that the child eats without chewing (P-Chewing; (B) items in the table; R indicates that the item is reversed).

TABLE 3 | Characteristics of the participating children ($N = 44$).

	Mean	**SD**	**Min**	**Max**
Height (cm)	139.5	9.0	126.1	167.6
Weight (kg)	33.2	7.1	23.8	54.5
BMI-for-age *z*-score	0.28	1.01	−2.17	2.42

males). Twenty-four children were followed by a dentist, and six had ongoing orthodontic treatment. They were on average 9.1 ± 0.9 years old and had, on average, a healthy weight status (**Table 3**).

The preloads were equally liked (apple segments = 8.1 ± 1.9, applesauce = 7.9 ± 1.6; $F(1, 87) = 0.3$, $p > 0.05$). The cottage cheese was equally liked ($F(1, 87) = 1.0$, $p > 0.05$) after apple segments (8.4 ± 1.7) or applesauce (8.6 ± 1.5).

Impact of Preload Form on Oral Exposure Time

The hypothesis that the preload form would affect oral processing duration was verified (**Table 4**): eating 77 g of apple segments required significantly more time than eating the same weight of applesauce [4.2 ± 1.6 min ($N = 40$) vs. 1.1 ± 0.4 min ($N = 42$), respectively; $t(37) = 12.20$, $p < 0.0001$]. The average difference in consumption duration between applesauce and apple segments was 3.1 ± 1.6 min (min = 0.9, max = 9.9 min). This is related to a longer oral exposure time, which reflects effective oral processing time, characterized by more bites, a smaller bite size, and a slower rate of eating (all $p < 0.05$, **Table 4**).

Effect of Preload Form on Hunger and *ad libitum* Intake

The average preload intake was 76.7 ± 2.1 g for the apple segments and 76.0 ± 0.7 g for the applesauce ($p > 0.05$). Children were quite hungry before preload consumption, and they reported being hungrier at the start of the applesauce snack (7.5 ± 1.9) than at the start of the apple segment snack (6.9 ± 2.0) ($F(1, 43) = 5.2$, $p < 0.05$; **Figure 2**). The hunger level significantly decreased over the course of the mid-afternoon snack regardless of the preload type consumed [time, $F(2, 215) = 115.47$, $p < 0.0001$; preload type $F(1, 215) = 9.07$, $p = 0.003$, but the time × preload type interaction was not significant ($p = 0.93$)]. Therefore, despite differences in hunger observed at the beginning of the snacks, the hunger decrease due to preload consumption was similar for both the applesauce and apple segment snacks (8.2 and 7.7%, respectively; $p > 0.05$) and was also similar after cottage cheese consumption (39.3 and 40.8% for the applesauce and apple segment snacks, respectively) ($p > 0.05$, **Figure 2**).

On average, 208 ± 120 g of cottage cheese was consumed after eating the apple segments, whereas 188 ± 107 g was consumed after eating the applesauce. We did not observe any effect of the preload type on *ad libitum* intake of cottage cheese afterwards, either when considering the cottage cheese alone ($p = 0.20$) or the total intake (apple preload + cottage cheese; $p = 0.19$) (**Table 5**).

In both sessions, cottage cheese intake was highly correlated with the observed eating rate ($\tau = 0.51$, $p < 0.0001$, $N = 37$ for the session with apple segments and $\tau = 0.50$, $p < 0.0001$, $N = 40$ for the session with applesauce). Thus, children who ate cottage cheese quickly had a higher *ad libitum* intake than those who ate at a slower rate. The eating rate of cottage cheese was consistent between both sessions ($N = 34$, $\tau = 0.50$, $p < 0.0001$).

TABLE 4 | Oral processing behavior variables (videotape coding) when eating both preload types and cottage cheese.

	Apple segments					Applesauce						
	N	Mean	SD	Min	Max	*N*	Mean	SD	Min	Max	*t* (df)**	*P*
Duration (s)	40	255	97	128	681	42	70	24	25	122	12.2 (37)	<0.0001
OralExposureTime (s)	37	227	90	113	629	42	68	24	25	122	10.6 (34)	<0.0001
Bite (*N*)	37	22	6	12	37	42	15	5	6	27	5.2 (34)	<0.0001
BiteSize (g/bite)	37	4	1	2	6	42	6	2	3	13	−5.2 (34)	<0.0001
EatingRate (g/min)	40	20	6	7	36	42	74	29	37	183	−14.3 (37)	<0.0001
MasticatoryCycles (*N*)	26	81	27	45	143							
***Ad lib* cottage cheese duration* (s)**	37	142	65	47	352	40	125	50	24	212	1.8 (33)	0.0860
***Ad lib* cottage cheese eating rate* (g/min)**	37	90	38	29	190	40	92	44	33	233	0.1 (33)	0.9033

**Values for the cottage cheese consumed after each preload (apple segments or applesauce); **t(df): Student's t-test.*

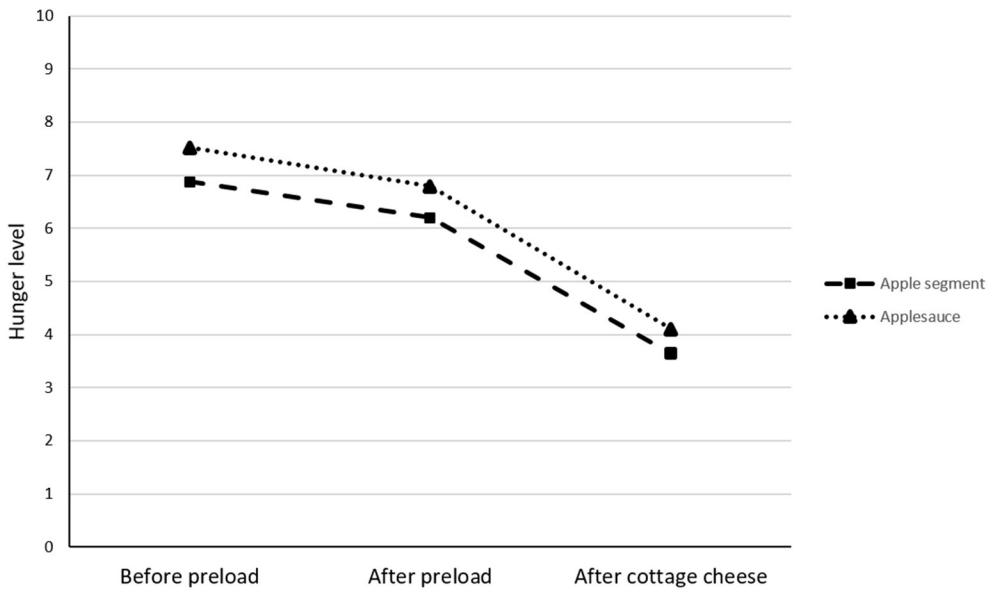

FIGURE 2 | Hunger ratings (mean ± SD) for the 44 children before and after preload and after cottage cheese consumption. The hunger level significantly decreased over the course of the mid-afternoon snack regardless of the preload type consumed (time, $F(2, 215) = 115.47$, $p < 0.0001$; preload type, $F(1, 215) = 9.07$, $p = 0.0029$), but the time × preload type interaction was not significant ($p = 0.93$).

Eating Rates: Links With Food Form and Individual Eating Temperament

We explored whether the eating rate observed for cottage cheese was consistent with those observed for the applesauce and apple segments. A significant correlation was observed between the eating rates of cottage cheese and applesauce ($N = 40$; $\tau = 0.44$, $p < 0.0001$) but not between the eating rates of cottage cheese and apple segments ($N = 37$; $\tau = 0.11$, $p = 0.32$).

Individual factors potentially influencing the preload eating rate were studied, including chewing behavior and eating temperament. A significant association between parental reports of children's chewing difficulties (P-noDiff_HardTexture) and the actual children's eating rate was observed in the case of apple segments ($F(2, 37) = 3.6$, $p = 0.04$) but not of applesauce ($p = 0.09$). No associations were observed between eating rate and parental report of children's chewing behavior before swallowing (P-Chewing) or any dimension of the CEBQ.

Further exploration revealed that children with chewing difficulties took significantly more time ($F(2, 37) = 5.3$, $p = 0.01$) to eat the apple segments than children with sometimes or without difficulties [6.0 ± 2.7 min ($n = 6$) vs. 4.1 ± 1.1 ($n = 7$) and 3.9 ± 1.1 min ($n = 27$), respectively]. They required significantly more bites (29.7 ± 6.5 bites vs. 21.9 ± 4.4 and 19.3 ± 4.4 bites; $F(2, 36) = 11.6$, $p < 0.001$) of smaller size (2.7 ± 0.7 g vs. 3.6 ± 0.8 and 4.1 ± 0.9 g, respectively; $F(2, 36) = 6.6$, $p = 0.004$).

DISCUSSION

This study investigated the impact of fruit form offered at the beginning of a mid-afternoon snack on reported hunger and

TABLE 5 | Weight and energy intake of cottage cheese after each preload (mean ± SD).

	Preload type	
	Apple segment	**Applesauce**
Cottage cheese intake		
Weight (g)	208 ± 120	188 ± 107
Energy (kcal)	192 ± 110	173 ± 99
Total intake (preload + cottage cheese)		
Weight (g)	285 ± 120	264 ± 107
Energy (kcal)	233 ± 110	214 ± 99

subsequent *ad libitum* intake of another food offered at the snack. Our results collected in healthy weight, school-aged children under controlled laboratory conditions showed, as expected, that eating apple segments took significantly more time and required more oral processing behaviors than eating the same quantity of applesauce matched for energy density. However, the main hypothesis of this work, based on a previous study in adults, was not confirmed: modifying the preload form (semisolid to solid) did not affect the level of hunger or the subsequent *ad libitum* intake of cottage cheese differently. In other respect, regardless of the preload, the cottage cheese *ad libitum* intake was highly correlated with the eating rate: fast eaters ate more during the snack. Nevertheless, children's eating rate was not consistent across foods: fast eaters of semisolid foods (cottage cheese and applesauce) were not necessarily fast eaters of hard foods, here raw apple segments. Moreover, child chewing difficulties as reported by their parents were negatively associated with the eating rate observed for chewable apple segments.

This study was inspired by a previous study in adults (11) and was adapted to children in the context of a mid-afternoon snack. The observed, expected effect of the apple form on oral processing behavior is consistent with a previous report in adults (38). For the raw apple segment, the eating rate was 20 g/min here (apple segment) vs. 27 g/min in Forde et al.'s study (entire apple); for applesauce, it was 74 g/min in our study vs. 90 g/min in Forde et al.'s study. Children have slower eating rates (26 and 18% lower for the apple segment and puree, respectively) than adults, but the decrease in eating rate due to food form is comparable for both populations.

However, the apple form-induced satiation observed in adults was not observed in our study involving 8- to 10-year-old children. Although a proper dedicated study would be necessary to draw firm comparisons between adults and children, some methodological differences between Flood-Obbagy and Rolls' study (11) and ours can be considered to discuss these contrasting results. These are related to the study protocol, meal context, and properties of the test food.

First, concerning the study protocol, Flood-Obbagy and Rolls (11) fixed the preload ingestion duration. We did not, as we hypothesized that the oral exposure time was the main factor influencing intake and that fixing the ingestion rate may be a less natural and disturbing situation for children. The break duration after preload consumption and the subsequent food intake were slightly different in both studies (15 min from the start of the consumption of the preload vs. 10 min after the consumption of the preload in our study). As the preload ingestion rate was left to the discretion of the child, fixing the break duration made it possible for us to strictly control the duration between the end of the preload consumption and the *ad libitum* intake for all children eating more or less quickly and for both preloads. However, in both cases, the break durations were not long enough to induce post-ingestive effects (30, 31).

Second, the study in adults was realized during a lunch (11), whereas we evaluated this in the context of a mid-afternoon snack, a much simpler meal in terms of number of dishes and in terms of different symbolic values of foods socially considered appropriate for a meal or a snack. Children's eating behaviors might be somehow slightly different between lunch and mid-afternoon snacks. Indeed, a French sociological exploration reported that although the mid-afternoon snack is considered a meal, it resists nutritional injunctions and is associated with the universes of sweetness and pleasure (39).

Third, concerning the test foods, the ED of the *ad libitum* food used by Flood-Obbagy and Rolls (11) is much higher [2.2 kcal/g for the cheese tortellini and tomato sauce (64% of energy from carbohydrate, 16% energy from fat, and 20% of energy from protein)] than that of the one we chose (0.92 kcal/g for the sweetened cottage cheese); thus, the satiating properties of these two foods might be quite different. Another explanation to consider is that the sweetened cottage cheese might have been slightly too much liked by the children. This food was chosen because it was relatively neutral, but it was revealed to be as liked as the two preloads. This might have increased its consumption slightly beyond satiation, especially in an *ad libitum* situation that might contrast with usual home practice when parents decide the size of the portion to eat. During the study, children were clearly happy to serve themselves.

Finally, we chose an *ad libitum* food that requires very little oral processing while the previous authors chose a more textured food requiring chewing behavior (pasta). One can hypothesize that a similar phenomenon as for sensory-specific satiation but specific to the textural properties (which could be named "texture-specific satiation") might explain why eating the apple segments first did not decrease the consumption of a cottage cheese, a food with different textural properties (and easier to eat from an oral processing perspective) than the food preload. Therefore, there may be a sensory control of the eating behavior for a succession of foods based on texture contrast. In this line, offering after a first food a second food with a different texture from the first one may reactivate the desire to eat. Not all mechanisms related to sensory-specific satiation have been understood so far. Nevertheless, sensory-specific satiation is known to be expressed differently in adults and children (40). Exploring this line of research is worthwhile to advance our understanding of the relation between texture perception (and oral processing capabilities) and appetite control abilities in children. Additionally, a better understanding of how children form expectations on satiating properties of a food based on texture is needed. In adults, as discussed by Nguyen and colleagues (41), it appears that "the effect of texture on satiety

expectations is not a straightforward function of hard/soft or viscous/not viscous, but rather related to a number of factors: viscosity, food particles, the complexity of the food items, their interaction, and their influence on the temporality of the in-mouth perception." Past experiences (i.e., familiarity) are also likely to be determinant (42): "children who ate the foods more often expected them to deliver greater satiation." Thus, our study clearly calls for more research to understand the links between food oral processing and satiation in children. In this context, other studies considering the strategy of "concurrent evaluation" of intake (while controlling for differences in food acceptance and familiarity as discussed earlier) would be of interest to complement the present results obtained with the preload paradigm. Indeed, direct measurements of *ad libitum* intake of foods bearing textural differences would provide complementary information on the role of food texture on consumption in children.

Our study allowed us to explore the variability in eating rate for the different foods in the child population. We observed that *ad libitum* cottage cheese intake was highly correlated with its eating rate, validating previous results in children showing that fast eaters eat more during a meal (18). In addition, eating rates of cottage cheese and applesauce were correlated, whereas they were not correlated with those of the raw apple segments. Therefore, fast eaters of semisolid foods may not necessarily be fast eaters for chewable foods. In addition, eating rate and, more generally, eating behavior in children should be seen in light of the development of their chewing skills, which might impact the role of food texture properties in satiation processes. Dental status is particularly important to consider. A previous study suggested that dental caries may affect eating habits (21, 22). Another study conducted in a Finnish interventional study [Special Turku Coronary Risk Factor Intervention Project (STRIP)] suggested a link between dental maturity, BMI, and energy intake in 148 children aged 6 to 12 years (43). Overall, this calls for more work to describe developmental changes related to food oral abilities during this very specific time frame and its effects on children's eating behavior. However, eating fast is thought to be a modifiable phenotype implicated in the overweight problem, but the links with oral processing capacities have not been explored much so far.

In conclusion, this pioneering study calls for further research to better understand the interplay between the textural properties of food, oral processing behaviors, food intake, and appetite control abilities in children. It is necessary to highlight new levers to explore to foster healthy food intake in children, whether or not they have chewing difficulties.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes Ile-de-France II N° IDRCB 2019-A00890-57. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

CS and CT designed the research with advice from SN. OP, CS, and CT conducted the research. ES performed the coding of all videotapes. CS and CT analyzed the data and wrote the paper. SN and OP critically revised the manuscript. All authors have primary responsibility for the content and approved the final draft.

FUNDING

This work was supported by grants from the Conseil Régional Bourgogne Franche-Comté (PARI grant) and the FEDER (European Funding for Regional Economical Development).

ACKNOWLEDGMENTS

The authors are grateful to Françoise Durey (ChemoSens Platform) for her help with recruitment. We thank Valérie Feyen and Chantal Septier for their help with the data collection. We also warmly thank the children and their parents who took part in this study.

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